STUDIES ON THE MECHANISM OF INDUCTION OF
PROPHAGE LAMBDA

by

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(a) Preliminary Observations on Induction

Recent research has been remarkably successful in elucidating many aspects of the biology of the bacteriophage lambda (\(\lambda\)). Consultation of the book of that name, edited by Hershey (1971), which is devoted in its entirety to this charismatic virus, reveals that much is known about its vegetative growth in the cell and that a promising start has been made towards understanding the control of the establishment of lysogeny. By contrast, little is known about the reversal of this process, the mechanism by which repression can be broken down in a lysogen. This is referred to as induction. It occurs spontaneously at a low frequency but it has been known for many years that treatment with numerous physical and chemical agents can greatly enhance this frequency.

These inducing agents (or treatments) include ultraviolet (UV) irradiation (Lwoff et al. 1950), X-rays (Latarjet 1951), mitomycin C (Otsuji 1959), thymine deprivation of thymine requiring lysogens (Korn and Weissbach 1962) and nalidixic acid (Cowlishaw and Ginoza 1970). Since most studies have used UV as the inducing agent and since its effects on the cell are better understood than the effects of other inducing treatments, I shall concentrate on this system in this survey. It should be noted that many, though not all, temperate phages are similar to \(\lambda\) in being inducible from the prophage state by agents such as those listed above.
In the lysogenic state, the \( \lambda \) prophage is physically integrated into the chromosome of *Escherichia coli*, where it is passively replicated *in situ*. Only a small region of the prophage is transcribed (Szybalski et al. 1970) which codes for the \( \text{cI} \) repressor and the \( \text{rex} \) gene product. The only known function of the latter product is the inhibition of growth of T4 \( \text{II} \) mutants on \( \lambda \) lysogens (Howard 1967).

The \( \text{cI} \) repressor binds to two operator sites located on either side of the region which is transcribed in the prophage (Ptashne and Hopkins 1968) preventing the expression of phage genes required for lytic growth in the cell.

Clearly, in order for the prophage to be induced, the \( \text{cI} \) repression system must be functionally inactivated. Theoretically this could happen in a number of ways. The inducing agent could act: (1) directly on the repressor or its target; (2) indirectly, by altering the cell in such a way that an inducer is produced which interacts with the repressor or (3) indirectly, by producing an inducer which alters the target of the repressor, e.g. by modifying its binding regions or by producing some structural change in the prophage.

Numerous lines of evidence rule out the possibility that UV directly inactivates the repressor and suggest that the first stage in UV induction is the introduction of UV photoproducts into DNA. For example, the UV action spectrum for induction (Franklin 1954) implicates DNA as the primary target, rather than protein whereas the repressor is known to be a protein (Ptashne 1967). Also the UV photoproducts which lead to induction are photoreactivable (Jacob and Wollman 1953; Tomizawa and Ogawa 1967) and several mutants defective in the repair of UV damaged DNA show modified inducibility (see section (c)). It is also unlikely that
inducing agents act directly on the repressor target since, as I shall discuss below, there is a time lag between treatment with the inducing agent and derepression.

The observation that mutations in the \( \sigma I \) gene are the only mutations mapping in the phage genome which are known to affect the inducibility of a lysogen without affecting subsequent phage growth, could be consistent with either of the last two possibilities outlined above. Two classes of mutants are known, \( \lambda^{\text{ind}^{-}} \) (Jacob and Campbell 1959), lysogens of which are not inducible by UV, and \( \lambda^{\text{ind}^{8}} \) (Horiuchi and Inokuchi 1967), which are more readily induced by UV than \( \lambda^{\sigma I^{+}} \). Following UV irradiation these mutations could alter the sensitivity of the repressor either to some change in another component of the immunity system (such as the operator sites) or to the direct action of the inducer.

Brief reports have been published recently which favour the second possibility, namely that inducing agents set in motion a sequence of events which culminate in the destruction of the repressor (Ptashne 1971; Reichardt et al. 1971). The first report cites unpublished data of Chadwick which are claimed to show that the DNA binding activity of the repressor is destroyed if cultures are irradiated with UV before the extracts are prepared. The second report states, in the form of an abstract, that after UV irradiation of a lysogen, the DNA binding activity of the repressor disappears and its antigenic activity changes as immunity is lost. In the absence of published data it is impossible to assess these reports critically.
It has been observed (Bertani 1958; Joyner et al. 1966) that early functions involved in lytic growth appear rapidly following either phage infection or thermal derepression of prophages which make temperature sensitive repressor (Sussman and Jacob 1962). In contrast, the appearance of these functions is delayed by 20-30 minutes if a lysogen is induced with, for example, UV or mitomycin C. Thus there is a significant time lapse between exposure of a lysogen to an inducing agent and derepression of the prophage.

Further evidence for this time lag can be found in a series of experiments by Tomizawa and Ogawa (1967). They utilised a derivative of λ, λb2 (Kellenberger et al. 1961) which carries a deletion extending into the phage attachment site (Fischer-Fantuzzi 1967). The phage is able to establish and maintain repression but is defective in integration. Thus, when it establishes repression following infection, its genome is not replicated but is passed unilinearly to only one of the daughter cells at division. By infection with λb2 and subsequent growth of the infected culture, it was possible to obtain a population of cells most of which no longer carried the phage genome but which still carried sufficient repressor to be immune to superinfecting λ or λcI. Using such populations of cells it was possible to demonstrate that either UV irradiation or treatment with mitomycin C could abolish their immunity to superinfecting phage. The full expression of the inducing effect of UV was found to require active growth for about 20 minutes after irradiation (or even longer periods for small UV doses). Evidence was also presented for a requirement for protein synthesis during
this period of growth if immunity was to be abolished, since the addition of chloramphenicol after irradiation prevented loss of immunity to infecting \( \lambda \text{ind} \).

One objection to these experiments is that they cannot necessarily be regarded as equivalent to the induction of an established integrated prophage and they may be investigating some effect of UV on the establishment of lysogeny by the infecting phage. The establishment of lysogeny is a complex process involving several competing phage gene products (Echols 1972), whereas induction probably involves only one phage gene product: the \( \text{cl} \) repressor. The observation that a \( \text{recA} \) mutation prevents the loss of immunity to an infecting phage in the system described above suggests that it bears some similarities to the induction of an established prophage, since a \( \text{recA} \) mutation prevents the latter but does not affect the frequency of lysogenisation (Brooks and Clark 1967). However, one would like to know the effect of pretreatment with chloramphenicol on the efficiency of lysogenisation of the infecting phage before one could accept unequivocally that UV induction requires protein synthesis. This is particularly important since there is evidence that induction in the following system does not require protein synthesis.

Noack and Klaus (1972) used a temperature sensitive mutant of \( E.\text{coli} \) of the \( \text{dnaB} \) type, which stops making DNA immediately at the restrictive temperature. The expression of the defect in this mutant also prevents the growth of \( \lambda \) (Lanka and Schuster 1970) presumably by affecting \( \lambda \) DNA replication. For this reason induction was not examined directly by looking for a burst of phage but by following the kinetics of loss of viability of a lysogenic derivative of this strain. A
comparison with the survival of the non-lysogen at the restrictive temperature showed that the lysogen was killed much more rapidly, suggesting that the prophage had been induced. This was observed whether or not chloramphenicol was present at the restrictive temperature. In a second series of experiments (Klaus et al. 1973) a λ lysogen of the same dnaB mutant was superinfected with a homoimmune phage (λbio N2-1 nin3) whose DNA had been labelled with ³H thymine. After superinfection, the cells were incubated at 30°C or at 42°C with or without chloramphenicol, lysed and the lysate fractionated by centrifugation through a neutral sucrose gradient. Where the cells had been incubated at 42°C the ³H label was associated with a fast sedimenting, presumably membraneous fraction, whether or not chloramphenicol was present. There was no increase in the association of ³H thymine with the fast sedimenting fraction at 42°C compared with 30°C if the prophage was λind⁻. This is good evidence that protein synthesis is not required for induction in this system since in the presence of repressor there should be no association of the superinfecting phage DNA with the membrane (Hallick et al. 1969).

Evidence has been presented above that the first stage in UV induction is the introduction of UV photoproducts into DNA. This raises the interesting question of whether the introduction of irradiated DNA into a lysogenic cell leads to induction. Borek and Ryan (1958) demonstrated that female lysogenic cells could be induced by mating with irradiated F⁺ cells, but that lysogenic F⁺ cells could not be induced by mating with irradiated females. The efficiency of this type of induction is increased if the donor cell carries a mutation
preventing the excision of UV damage from its DNA (Devoret et al. 1965; George and Devoret 1971). Furthermore, induction does not occur if the lysogenic recipient can restrict the DNA of the male donor (George 1966). This suggests that induction can result from the introduction of UV irradiated DNA into a lysogenic cell, a phenomenon termed indirect induction. The similarity of indirect to direct induction, in which the inducing treatment is applied directly to the lysogen, is established by the observation that directly inducible phages (e.g., λ 434) can be induced indirectly whereas the non-inducible prophage λind− cannot (Devoret and George 1967). Furthermore, recA lysogenic recipients cannot be induced either directly or indirectly (Brooks and Clark 1967; George and Devoret 1971).

The ColI factor has also been shown to mediate indirect induction (Monk and Devoret 1964). However, the introduction of irradiated DNA into a lysogen is not sufficient per se to cause induction. First, DNA transferred from UV irradiated Hfr cells does not induce indirectly with high efficiency (Devoret and George 1967). Second, a number of irradiated phages such as λimm434, φ80, T6 (Rosner et al. 1968), φX174 and M13 (Monk 1969) do not indirectly induce. Third, interrupted mating experiments using a series of large F-prime factors, in which the time of entry of the complete sex factor was measurably delayed by the large component of bacterial genes carried by the episome, showed that indirect induction did not occur until all of the bacterial genes carried on the episome had been transferred (Rosner et al. 1968). These results suggest that elements capable of mediating indirect induction are replicons which can stably exist in
a cell without integration into the host chromosome. This hypothesis is supported by the demonstration that irradiated phage PI can induce λ prophage (Rosner et al. 1968). It is known that PI can form stable lysogens without integration into the chromosome (Ikeda and Tomizawa 1968) and is thus inherited in a stable extrachromosomal manner like F and ColI factors.

A further observation made by Rosner et al. (1968) was that the inducing effect of an irradiated replicon can be blocked by the pre-existence in the recipient lysogen of a replicon of the same type. When F⁺ or Hfr lysogens are converted into F⁻ phenocopies they cannot be induced by transfer of an irradiated F into them, although they remain inducible by irradiated PI. Conversely, lysogens carrying both λ and PI are inducible by irradiated F⁺ but not by irradiated PI.

There has been a recent report that phage Mu-1 can also induce λ (Kanter and Harriman 1972), although the UV doses used were far higher than those normally employed when studying indirect induction. Until the effect of irradiated Mu-1 on non-inducible systems such as λind⁻ lysogens or recA (λ) lysogens has been determined this result should be treated with caution. If the ability of irradiated Mu-1 to induce is confirmed, it will be necessary to investigate whether prophage Mu-1 can exist, under certain circumstances, in the autonomous rather than integrated state. The observation that some E.coli mutants resistant to PI are also resistant to Mu-1 (Taylor and Trotter 1972) may suggest a relationship between these two phages. If, however, Mu-1 is always integrated in the prophage state, it will be necessary to revise our model about indirect induction.

-8-
To summarise the points established thus far. First, UV induction begins with the introduction of UV photoproducts into DNA, and ends after a minimum of about 20 minutes in the derepression of the prophage. This derepression is probably achieved by the destruction of the CI repressor. Second, UV induction may require protein synthesis, but the evidence indicates that derepression following incubation of a dnaB mutant at the restrictive temperature does not. Third, induction may also be achieved by introducing an irradiated replicon, which has the potential for stable extrachromosomal existence into a lysogenic recipient which does not already harbour a replicon of the same type.

(b) The Physiological Approach to Induction

One approach to the elucidation of the mechanism of induction is to examine the metabolism of non-lysogenic cells after treatment with an inducing agent and to investigate whether any alterations detected play a role in induction.

The observation that inducing treatments are almost invariably specific inhibitors of DNA synthesis has frequently led to the suggestion that these two phenomena are connected (for example, see Goldthwait and Jacob 1964; Bonhoeffer and Messer 1969; Worcel 1970; Noack and Klaus 1972).

Recent evidence, however, (Monk and Gross 1971) suggests that one can inhibit DNA synthesis without causing induction in lysogens which retain the potentiality for induction by UV irradiation. These experiments involved a dnaA mutant of E.coli which is thought to be temperature sensitive for the initiation of rounds of DNA replication.
On placing such a mutant at 42°C net DNA synthesis stops after its DNA content has increased by the amount which would be expected if rounds of replication already initiated were completed. It was reported that no spontaneous stimulation of phage production was observed when a lysogenic derivative of a dnaA mutant was incubated at 42°C, but that phage production could be elicited by irradiation after 75 minutes at 42°C, as measured by stimulation of net DNA synthesis and the production of infectious phage particles.

Attempts have also been made to examine DNA metabolism in non-lysogenic cells under conditions favouring indirect induction. Monk (1969) investigated the effect of transferred irradiated ColI DNA on DNA synthesis in a non-lysogenic recipient. She reported no consistent effect although the data she published did suggest some decrease in the rate of host DNA synthesis. Wilkins and Hollom (1972) made a rigorous investigation which supported Monk's claim. One interesting observation made by Monk (1969) was that transfer of the radiation damaged Col factor into non-lysogenic cells could lead to the production of filaments. This observation will be discussed in the final section.

(c) The Genetic Analysis of Induction

Another approach to the elucidation of the mechanism of induction has been to analyse bacterial mutants with altered induction properties. Devoret and Blanco (1970) have described a method for the isolation of non-inducible mutants. They have so far published only a preliminary analysis of the mutants obtained (Devoret et al. 1972) which will be
discussed further below. Most of the other mutations which have been shown to effect induction, however, were originally isolated as conferring other phenotypic properties.

It was previously argued that the introduction of UV photoproducts into DNA is the first stage in UV induction. It is therefore of interest to investigate whether mutants defective in the repair of UV damage show modified inducibility. DNA repair is a complex field and for detailed information and documentation on matters discussed here, the reader is referred to reviews by Howard-Flanders (1968), Witkin (1969) and Clark (1971).

Excision repair of UV damage, which was the first dark repair system to be described in _E. coli_, involves the recognition of an area of DNA containing a pyrimidine dimer, the principal photoproduct induced in DNA by UV irradiation, followed by localised endo- and exonucleolytic action to remove a portion of the DNA strand containing the damaged nucleotides. The excised region is then resynthesised by a DNA polymerase which uses the intact complementary strand as a template. Mattern _et al._ (1965) and Monk _et al._ (1971) have shown that mutants defective in this repair process show increased inducibility at small UV doses. Presumably this indicates that UV induction requires the continued presence of excisable photoproducts in DNA.

In addition to mutants defective in excision repair, several other UV sensitive mutants are known which may be defective in repair processes. Some of these show either reduced or no induction of prophage λ following UV irradiation. Some of the phenotypic characteristics conferred by the most studied of these mutations, those in the _recA_ and _lex_ genes, are
summarised in Table 1. An $\text{exrA}$ mutation of the type described in $\text{E.coli} \text{~B}$ and a $\text{lex}$ mutation of the type described in $\text{E.coli} \text{~K12}$ confer comparable phenotypes, are similar in map position, and probably represent mutations in genes specifying similar gene products (Witkin 1969b).

Lysogens carrying a $\text{recA}$ mutation show a negligible level of both spontaneous and UV induced phage production (Brooks and Clark 1967; Hertman and Luria 1967), whereas both types of phage production are reduced to about 10% of the wild type levels in lysogens mutant at $\text{exrA}$ (Donche et al. 1970; 1971).

Attempts have been made to explain the non-inducibility of $\text{recA}$ mutants and the poor inducibility of $\text{exrA}$ mutants on the basis of their other properties. However, since both are highly pleiotropic mutants and the nature and primary functions of their gene products are unclear, this is not a simple task.

Other UV sensitive mutants have been described which block induction. One of these, mutant at a gene originally designated $\text{uvrf}$ (Storm and Zaunbrecher 1972) was reported to be highly sensitive to the lethal effect of UV irradiation, though only slightly sensitive to X-rays. It degrades its DNA considerably more than its parental strain after UV irradiation and shows no spontaneous or UV induced production of phage.

The $\text{uvrf}$ gene has now been designated $\text{recF}$, the designation being taken from mutants described by Horii and Clark (cited in Taylor and Trotter 1972).

In addition to $\text{recA}$, $\text{lex}$ and $\text{recF}$ mutants, a temperature sensitive mutant strain designated T44 has been isolated in which UV
<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>A mutation at recA</th>
<th>A mutation at lex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confers inability to form genetic recombinants [1,2,3]</td>
<td>Has little, if any, effect on the ability to form genetic recombinants [8,9]</td>
<td></td>
</tr>
<tr>
<td>Confers enhanced sensitivity to UV and X-irradiation [1,2]</td>
<td>Confers enhanced sensitivity to UV and X-irradiation [10]</td>
<td></td>
</tr>
<tr>
<td>Confers a tendency to degrade DNA, both spontaneously and particularly after irradiation [2,4]</td>
<td>Confers a tendency to degrade DNA, both spontaneously and particularly after irradiation [9,10]</td>
<td></td>
</tr>
<tr>
<td>Confers the ability to divide during thymidine starvation of a thymine-requiring mutant [6]</td>
<td>Prevents UV mutagenesis [12]</td>
<td></td>
</tr>
<tr>
<td>Prevents UV mutagenesis [7,8]</td>
<td>Prevents UV reactivation [7]</td>
<td></td>
</tr>
</tbody>
</table>

1. Clark and Margulies (1965)  
2. Howard-Flanders and Theriot (1966)  
3. Low (1968)  
4. Clark et al. (1966)  
5. Green et al. (1969)  
6. Inouye (1971)  
7. Miura and Tomizawa (1968)  
8. Witkin (1969a)  
9. Mount et al. (1972)  
10. Howard-Flanders and Boyce (1966)  
11. Donch et al. (1968)  
12. Witkin (1967b)  
13. Defais et al. (1971)
inducible prophages (e.g. λ, 424, 434, 21) are only stable at low temperatures, whereas non-inducible prophages (e.g. 18, 299, λind^-) are stable at all temperatures (Goldthwait and Jacob 1964; Kirby et al. 1967). The mutation in this strain has been shown to map very close to recA and has been designated tif-l (Castellazzi et al. 1972a). The observation that only UV inducible prophages can be thermally induced in T44 suggests that some effect is produced in this strain at the non-permissive temperature which is similar to some stage in the process of UV induction.

An interesting feature of this mutant is that its inducibility can be profoundly modified by the addition of various nucleosides and purine and pyrimidine bases to the growth medium. For example, adenine promotes induction, even at the permissive temperature, whereas guanosine and cytidine can largely prevent induction even at the non-permissive temperature (Kirby et al. 1967). Attempts have been made to investigate the levels of common purine and pyrimidine derivatives in both T44 and its parental strain (C600) under inducing and non-inducing conditions, but no major differences have been observed (Ruff et al. 1971). A number of other low molecular weight compounds all of which have a five membered oxygen containing ring have now been shown to promote or inhibit induction in this strain (Kirby et al. 1972). Interestingly evidence has been presented that under some conditions certain temperature sensitive repressors cannot be heat inactivated except in the presence of some metabolite capable of being synthesised in starved cells following the addition of nucleosides (Butcher and Green 1969). This might mean that some low molecular weight compound derived from nucleosides can affect the stability of the repressor. If so, in T44 which is rather delicately balanced
between induction and repression, the effect of the low molecular weight compounds may be to combine with the repressor and shift the balance towards or away from induction.

When T44 was cured of its prophage and its metabolism examined under inducing conditions, it was found that cell division was inhibited and the cells formed long non-septate filaments (Kirby et al. 1967). Unfortunately, no data were published on the kinetics of this process.

Clearly, it is of great importance to know whether DNA metabolism is affected in some way in the non-lysogenic strain under inducing conditions. Such an effect might explain both the prophage induction and the inhibition of cell division, since as will be discussed in the next section, there is evidence that cell division is intimately related to the DNA replication cycle. However, it has been claimed that DNA synthesis is not affected in the non-lysogen at the restrictive temperature, that nuclear material is distributed throughout the filaments and no DNA degradation or strand breakage is detectable (Kirby et al. 1967). Furthermore, the expression of the mutation in T44 has no effect on the ability of T44 to donate DNA during conjugation and transferred (unirradiated) F DNA does not cause indirect induction in a lysogenic recipient (Castellazzi et al. 1972a).

The expression of the defect in T44 leads to another interesting effect apart from an inhibition of cell division, namely an enhanced survival and mutagenesis of infecting irradiated phage λ (Castellazzi et al. 1972a).

Three classes of revertants have been isolated which suppress induction, filamentation and the enhanced ability to repair phage DNA
in T44 (Castellazzi et al. 1972b). These include recA and lex mutants already discussed, and a new class zab which map very close to both tif-1 and recA.

Mutants defective at zab were found to be highly sensitive to UV irradiation, X-rays and mitomycin C and to degrade their DNA drastically after UV irradiation. They are also deficient in the repair and mutagenesis of UV irradiated phage λ and show a very low spontaneous production of phage λ. In these properties zab mutants are similar to recA mutants, but in contrast they are little, if at all, recombination deficient, they are slightly inducible by UV or mitomycin C and they do not degrade their DNA spontaneously (Castellazzi et al. 1972b).

The non-inducible mutants described by Devoret et al. (1972) fall into at least four classes: recA, Itd-1, Itd-2 and Itd-3. Devoret et al. (1972) suggested that Itd-3 mutants were similar to lex mutants. However, the failure of these workers to locate the lesion in Itd-3 mutants near to malB or meta by transduction and the very low level of spontaneous induction shown by these mutants suggest that they may be more closely related to zab mutants (Castellazzi et al. 1972b). Itd-2 mutants do not permit the growth of lamboid phages (Devoret et al. 1972) suggesting that they may not allow the development of the prophage subsequent to derepression rather than affecting induction.

Itd-1 mutants are more interesting. They are non-inducible by either UV irradiation or thymine deprivation. Non-lysogenic Itd-1 derivatives are as resistant as wild-type to the killing action of UV or X-irradiation and chromosome mobilisation with an F-prime factor is
unimpaired. This suggests that they are not deficient in either their DNA repair or recombination abilities. They degrade their DNA less than their parental strain after thymine starvation but more after UV irradiation. Finally, whereas wild-type cells elongate after UV irradiation and form snakes after thymine deprivation, neither treatment leads to filament formation in Itd-1 bacteria (Devoret et al. 1972).

(d) Some Possible Mechanisms of Induction

(1) DNA degradation and the accumulation of nucleotides.

It has been postulated that induction could result from the accumulation of DNA precursors following an inhibition of DNA synthesis (Goldthwait and Jacob 1964; Hertman and Luria 1967; Noack and Klaus 1972). However, the observation that one can block or at least greatly reduce DNA synthesis without inducing a prophage in cells which retain the potentiality for induction (Monk and Gross 1971) argues against this hypothesis. Strong evidence against it also comes from studies on indirect induction which show that a transferred irradiated replicon does not inhibit host DNA synthesis (Monk 1969; Wilkins and Hollom 1972).

Hertman and Luria (1967) suggested that recA mutants were non-inducible because of their tendency to degrade their DNA excessively, both spontaneously and in particular after UV irradiation. The mutants described in the previous section as showing reduced or no ability to be induced each had a tendency to degrade their DNA to some extent after UV irradiation. However, there is no simple correlation between DNA breakdown and inducibility since both recA
mutants which degrade their DNA excessively and recA recB mutants which degrade their DNA less than rec+ cells after UV irradiation are non-inducible (Willetts and Clark 1969). It should be pointed out that breakdown products are likely to vary biochemically depending on the nature of the nuclease producing them. Consequently this observation does not rule out a role for some specific product of DNA degradation in either promoting or inhibiting induction. However, if one postulates this, one has to explain why only certain irradiated replicons have so far been shown to cause indirect induction. The hypothesis would imply that not all types of damaged DNA are degraded in the same way. A hypothesis linking DNA breakdown and induction can be regarded as a special case of the hypothesis linking induction with DNA repair and in future discussions will not be considered separately from the latter hypothesis.

(2) DNA repair.

The four types of mutants recA, lex, zab and recF which, as described in Section c, can be UV induced poorly if at all, are all more sensitive to the lethal effects of UV irradiation than their parental strains. This suggests that they are defective in some DNA repair process. In addition, a fifth mutant in which induction is affected, T44, has an enhanced capacity to repair UV damaged phage DNA under inducing conditions. A reasonable hypothesis to explain induction might, therefore, be that the repressor recognises some product of a repair system.

The difficulty with such an hypothesis is again the results from experiments on indirect induction. One has to explain why the
repair system involved in induction only acts efficiently on certain types of irradiated replicons. This hypothesis will be considered in more detail in Chapters VI and VII.

(3) **Initiation of rounds of DNA replication**

Yoshikawa and Haas (1968) and Worcel (1970) have suggested that there may be a common regulatory substance involved in both the initiation of rounds of DNA replication and prophage induction. This hypothesis fits well with the observation that only replicons with the ability to coordinate their replication with the host cell cycle are able, when irradiated, to induce indirectly.

Their models predict that the hypothetical regulatory substance should accumulate following the inhibition of DNA synthesis. It is difficult to reconcile them with the data of Monk and Gross (1971) which show that one can inhibit DNA synthesis without inducing a prophage in cells which retain the potentiality, when irradiated, to be induced. However, this experiment involved the use of a lysogen carrying an ill-understood mutation which itself prevents the initiation of rounds of DNA replication. The expression of the mutation in this strain may affect the accumulation of the hypothetical regulatory substance and it is possible that UV irradiation may exert some effect on the lysogen under these conditions, such as the promotion of DNA degradation or the temporary inactivation of a gene which in turn may affect the accumulation of the regulatory substance.

(4) **Inhibition of cell division**

Witkin (1967a) has listed a number of observations suggesting that there may be a relationship between filamentation in *E. coli* B and prophage induction. Most of the points she lists suggest that
both result from disturbances to DNA metabolism. It has been demonstrated that cell division stops shortly after treatment with inhibitors of DNA synthesis such as UV irradiation, nalidixic acid or mitomycin C and it has been demonstrated that cells which divide in the presence of such inhibitors correspond with cells which would have completed a round of DNA replication at the time of treatment with the inhibitor (Clark 1968a; Helmstetter and Pierucci 1968).

Some of the observations which suggest that there may be an intimate relationship between prophage induction and the inhibition of cell division are the following.

First, there is the behaviour of the mutant T44, described above, in which both cell division and induction are affected by a single mutation, despite the fact that no defect has been observed in the DNA metabolism of this mutant.

Second, mutations in three separate genes, recA, lex and zab, has been found to suppress both filamentation and induction in this mutant (Castellazzi et al. 1972b). Two of these mutations, recA and lex, have also been shown to suppress filamentation in a lon mutant following exposure to UV irradiation (Donch et al. 1968; Green et al. 1969). A lon mutation renders the division apparatus of the cell highly sensitive to inhibitors of DNA synthesis such as UV so that a lon mutant readily forms filaments following exposure to such agents (Adler and Hardigree 1964; Howard-Flanders et al. 1964). Thymine requiring recA mutants can also continue dividing when starved for thymidine or after treatment with nalidixic acid, both of which lead to inhibition of division in rec+ cells (Inouye 1971). It has
also been reported that Itd-1 mutants, which are non-inducible by UV or thymine starvation, do not elongate or form filaments following UV irradiation or thymine starvation (Devoret et al. 1972).

Third, there is the observation that a transferred irradiated ColI factor can cause filamentation in a non-lysogenic recipient (Monk 1969). Thus, although irradiated replisomes which cause indirect induction may not inhibit host DNA synthesis, there is evidence that at least the irradiated ColI factor can inhibit division.

Fourth, there are the observations that λ forms clear plaques on lon mutants on some media, due to a reduced frequency of lysogenisation and that the level of spontaneous induction is higher in lon than in lon+ lysogens (Walker et al. 1973). These workers suggest that the lon mutation, which as described above affects cell division, may also affect the synthesis or function of the ci repressor. However, the relationship of this observation to UV induction, if any, has not been established. Kirby et al. (1967) have reported that lon lysogens can form filaments at low UV doses without phage production, although the latter can be elicited by UV doses of the magnitude required for UV induction of lon+ lysogens.

Kirby et al. (1967) also reported that treatment with penicillin G or crystal violet caused filament formation in several strains without causing phage production. Furthermore, Hirota et al. (1968) have described several mutants which, like T44, are temperature sensitive for septum formation but in which prophage development is not induced at the high temperature. This suggests that there is no direct causal relationship between prophage induction and the inhibition of
cell division but leaves open the possibility that the explanation
for induction may be found in the events leading up to the inhibition
of cell division following treatment with agents such as UV.

Initial Experimental Objectives

Some possible explanations for the mechanism of prophage induction,
together with some objections to them, have been considered. Further
discussion of the hypothesis that induction is caused by the action of
a repair system will be deferred until subsequent chapters, where it
will be evaluated in the light of experimental results obtained.

The hypothesis that induction is caused by the accumulation of a
regulatory substance involved in the initiation of a round of DNA
replication suggests that lysogens of different ages should respond
with varying efficiencies to induction by UV. The same will be true
of the hypothesis that induction is related to the inhibition of cell
division if it is assumed that division can be more readily inhibited by
irradiation at one stage in the division cycle than another. This will
be discussed more extensively in Chapter IV.

The concept that cells of different ages do not show the same
sensitivity to inducing treatments receives support from experiments
on induction using nalidixic acid (Cowlishaw and Ginoza 1970). Nalidixic
acid is reputed to be a specific inhibitor of DNA synthesis (Goss et al.
1965) the effects of which are rapidly reversible by dilution of the
culture out of the drug. By adding it in varying concentrations to
lysogenic cells and diluting them free of it at varying times thereafter,
the kinetics with which cells were irreversibly switched into the
induced state were studied. It was found that at all concentrations of drug some cells were immediately switched into the induced state but that the rate with which cells became switched depended upon the concentration of the drug added. It was claimed that since the drug inhibited DNA synthesis almost immediately after its addition to the culture, the fact that some cells became immediately irreversibly induced, whereas others required continued growth in the presence of the drug for induction to occur, constituted evidence for a heterogeneity in the response of the population. It was suggested that this might be due to a heterogeneity in the stage of the division cycle in an asynchronous population of cells.

This is an attractive explanation, although the data are susceptible to other interpretations. For example, it was suggested in Section 2 that UV might lead to induction for reasons other than the fact that it inhibits host DNA synthesis. If this were so then a possible explanation for the experiment outlined above would be a gradual accumulation of damage in the DNA as the cells are exposed to nalidixic acid for longer periods of time. The mechanism of action of nalidixic acid is uncertain, but it is known to be bactericidal (Goos et al. 1964); it can lead to DNA degradation (Cook et al. 1966a; Kantor and Deering 1968); it may be mutagenic (Cook et al. 1966b); and it kills recA, recB and excA mutants more readily than rec+ exc+ strains (Green et al. 1970). It is possible therefore that nalidixic acid does lead to damage of the DNA.

Although the evidence to suggest that induction occurs as a consequence of a disturbance to the cell cycle is clearly equivocal, it warrants further investigation. I therefore set out to test the
essential prediction of this hypothesis which is that cells at
different stages in the cell cycle should show a variation in their
ability to be induced.
CHAPTER II
MATERIALS AND METHODS

Bacterial Strains

All of the bacteria used in experiments described in this Thesis are strains of *E.coli*. The following strains were used (lysogenic derivatives are generally not listed):

<table>
<thead>
<tr>
<th>STRAIN</th>
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<th>SOURCE</th>
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</thead>
<tbody>
<tr>
<td>C600</td>
<td>F&lt;sup&gt;-&lt;/sup&gt;, thi-1 thr-1 leu-6</td>
<td>R. Devoret (A101)</td>
</tr>
<tr>
<td></td>
<td>supE44 lacY1 tonA21</td>
<td></td>
</tr>
<tr>
<td>C600 Sm&lt;sup&gt;R&lt;/sup&gt;</td>
<td>C600 str</td>
<td>R. Devoret (A102)</td>
</tr>
<tr>
<td>C600 Pen&lt;sup&gt;R&lt;/sup&gt;</td>
<td>C600 ampA</td>
<td>B.M. Wilkins</td>
</tr>
<tr>
<td>P4X</td>
<td>Hfr, metB1 rel-1</td>
<td>B.M. Wilkins</td>
</tr>
<tr>
<td>B/r</td>
<td>malB</td>
<td>W. Vielmetter</td>
</tr>
<tr>
<td>B/r mal&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
<td>Mal&lt;sup&gt;+&lt;/sup&gt; transductant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Donor: B251)</td>
</tr>
<tr>
<td>B/r (λ)</td>
<td></td>
<td>λ lysogen of B/r mal&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>B251</td>
<td></td>
<td>Arber and Dussoix (1962)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>via S. Glover.</td>
</tr>
<tr>
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<td>B251 str</td>
<td>Mutant of B251</td>
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<td>trp malB uvrA</td>
<td>B. Bridges</td>
</tr>
<tr>
<td>WP2 hor mal&lt;sup&gt;+&lt;/sup&gt;</td>
<td>trp uvrA</td>
<td>Mal&lt;sup&gt;+&lt;/sup&gt; transductant</td>
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<td>trp</td>
<td>Mal&lt;sup&gt;+&lt;/sup&gt; Hor&lt;sup&gt;+&lt;/sup&gt; transductant</td>
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<tr>
<td>W3350</td>
<td>sup&lt;sup&gt;+&lt;/sup&gt;</td>
<td>C. Radding (B206)</td>
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<td>F-, recA1 dra str</td>
<td>Thy+ recombinants from W3110 x KL16-99</td>
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<td>F-, dra str</td>
<td></td>
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<tr>
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<td>F-, xyl end</td>
<td>B. M. Wilkins</td>
</tr>
<tr>
<td>MC14</td>
<td>F-, dnaA83 lac thr leu thi thyA dra drm</td>
<td>M.G. Chandler</td>
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**Phage Strains**

The following phage strains were used in the experiments described in this Thesis:

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<th>STRAIN</th>
<th>SOURCE</th>
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<td>(\lambda) ref ((\lambda))</td>
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</tr>
<tr>
<td>(\lambda) vir</td>
<td>R. Devoret</td>
</tr>
<tr>
<td>(\lambda) c26</td>
<td>R. Devoret</td>
</tr>
<tr>
<td>(\lambda) cI857 ind”</td>
<td>R. Devoret</td>
</tr>
<tr>
<td>(\lambda) cIus6 Nsus7sus53</td>
<td>M. Lieb</td>
</tr>
<tr>
<td>(\lambda) imm434</td>
<td>R. Devoret</td>
</tr>
<tr>
<td>(\lambda) imm434 ind”</td>
<td>See this Chapter</td>
</tr>
<tr>
<td>Plvl</td>
<td>B. M. Wilkins</td>
</tr>
</tbody>
</table>

**Media**

\(S2\): 6g \(\text{Na}_2\text{HPO}_4\); 3g \(\text{KH}_2\text{PO}_4\); 1g Difco vitamin free casamino acids; 0.5g \(\text{NaCl}\); 0.25g \(\text{MgSO}_4\cdot7\text{H}_2\text{O}\); 0.1g \(\text{NH}_4\text{Cl}\); 11mg \(\text{CaCl}_2\); 2mg thiamine; 5g glucose per litre of distilled water.

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SL: Same as S2 but 0.5g glucose instead of 5g per litre.

HC: Helmstetter (1967). 6g Na\textsubscript{2}HPO\textsubscript{4}; 3g KH\textsubscript{2}PO\textsubscript{4}; 3g NaCl; 2g NH\textsubscript{4}Cl; 0.25g MgSO\textsubscript{4}.7H\textsubscript{2}O per litre of distilled water.

Each litre of HC minimal salts was supplemented as appropriate with 2g casamino acids, 1g glucose and 50mg L-tryptophan or 1g glucose, 50mg L-methionine and 50mg L-histidine or 1g glucose or 0.4g L-proline and 0.4g L-alanine or 5.5g sodium succinate.

HCTC: 1g glucose; 0.5g sodium citrate; 20mg L-tryptophan per litre of HC minimal salts.

Nutrient broth (NB): 25g Oxoid No.2 nutrient broth powder per litre of distilled water.

Tryptonebroth (TB): 10g Oxoid tryptone; 5g NaCl per litre of distilled water.

TBMM: 10g Difco-Bacto tryptone; 5g NaCl; 2g maltose; 2.5g MgSO\textsubscript{4}.7H\textsubscript{2}O per litre of distilled water.

Luria broth: 10g Oxoid tryptone; 5g Difco yeast extract; 5g NaCl per litre of distilled water (pH adjusted with NaOH to 6.8-7.2).

C medium: 6g Na\textsubscript{2}HPO\textsubscript{4}; 3g KH\textsubscript{2}PO\textsubscript{4}; 1g NH\textsubscript{4}Cl; 0.5g NaCl; 0.25g MgSO\textsubscript{4}.7H\textsubscript{2}O; 20mg L-threonine; 20mg L-leucine; 11mg CaCl\textsubscript{2}; 1mg thiamine; 4g glucose per litre of distilled water.

Nutrient agar (NA): 25g Oxoid No.2 nutrient broth powder; 12.5g Davis agar per litre of distilled water.

Minimal agar: 15g Davis Japanese agar; 6g Na\textsubscript{2}HPO\textsubscript{4}; 3g KH\textsubscript{2}PO\textsubscript{4}; 1g NH\textsubscript{4}Cl; 0.5g NaCl; 0.25g MgSO\textsubscript{4}.7H\textsubscript{2}O; 11mg CaCl\textsubscript{2}; 10mg thiamine; 1g glucose per litre of distilled water.

Lambda Tryptone agar (LTA): 10g Difco-Bacto tryptone; 5g NaCl; 10g Oxoid No.1 agar per litre of distilled water.
Z agar: 10g Oxoid No.1 agar; 0.37g CaCl$_2$; 1g glucose per litre of nutrient broth.

Z soft agar: 6g Oxoid No.1 agar; 0.37g CaCl$_2$ per litre of nutrient broth.

Lambda soft agar (LSA): 10g Oxoid tryptone; 7g Oxoid No.1 agar; 5g NaCl per litre of distilled water. MgSO$_4$ was added to $10^{-2}$M before use.

Phosphate Buffer (PB): 7g Na$_2$HPO$_4$; 4g NaCl; 3g KH$_2$PO$_4$; 0.1g MgSO$_4$·7H$_2$O per litre of distilled water (pH 6.9).

Lambda Buffer (LB): 2.5g MgSO$_4$·7H$_2$O; 50mg gelatin; 0.73g Tris per litre of distilled water (pH 7.2).

Scintillation Fluid: 5g PPO (2,5-diphenyloxazole); 0.3g dimethyl POPOP (1,4-BIS-2-[4-methyl-5-phenyloxazolyl]-benzene) per litre of toluene.

**Phage Preparation**

Phage were prepared either by UV induction of lysogens or by plating about $10^6$ plaque-forming units (pfu) of phage with an appropriate indicator strain. In the latter case the soft agar was scraped from the plates after 6 hours, transferred to a tube, mashed and incubated with chloroform for 15 min. After centrifugation, the upper aqueous layer was decanted, residual chloroform removed by aeration, and the lysate stored at 4°C.

**Indicator Bacteria and Phage Titration**

Cells were inoculated into TB and grown overnight without aeration. The culture was centrifuged and the cells were resuspended in LB at 1-2 x $10^9$ cells/ml. They were shaken at 37°C for 1 hour and the starved cells were stored at 4°C and used for up to 5 days.
from the day of preparation. Phage were allowed to adsorb to indicator
cells for 20 min at 37°C before plating in LSA on LTA plates.

**Lysogenisation**

A strain to be lysogenised was plated in LSA and phage was
spotted onto the surface of the soft agar. After overnight incubation
cells from the area of lysis were streaked onto an NA plate. Isolated
colonies were restreaked to single cells, and exponentially growing
cultures derived from the resulting colonies were tested for lysogeny
by streaking over λvir and λc26 or by streaking such cultures over an
appropriate indicator strain plated in soft agar.

**UV Irradiation**

For UV irradiation, cells or phages were suspended in buffer and
irradiated in a layer no more than 2mm deep under an Hanovia germicidal
lamp. The dose rate was adjusted using a Latarjet dosimeter (Latarjet
et al. 1953). Irradiated cells and cells infected with irradiated
phages were maintained in subdued light for at least 2½ hours after
irradiation or infection to prevent photoreactivation.

**Infective Centres Assay for UV Induction**

Following irradiation, lysogens were diluted in LB and plated
with Sm^R indicator bacteria in 3.3ml LSA. The plates were incubated
at 37°C for 2½ hours, then 2ml LSA containing 1.2 mg of streptomycin
was added. The plates were incubated overnight at 37°C. It is
assumed that each induced lysogen gives rise to a plaque under these
conditions and by measuring the number of lysogens used in each
experiment, the fraction induced by a given UV dose can be calculated.
When the lysogen was Sm$^R$ the technique was modified by using a Pen$^R$ indicator strain and incorporating penicillin G at 100 μg/ml in the LTA plates and the LSA.

**PI Transduction**

A lysate of phage P1vl was prepared on a suitable donor strain, using the plating method described above and Z top and bottom agar. Phages were added at a multiplicity of infection of about 0.1 to a culture of recipient cells growing exponentially in Luria broth supplemented with CaCl$_2$ (2.5 mM). The mixture was incubated at 37°C for 25 minutes then the cells were washed and resuspended in PB containing 0.25% sodium citrate. Aliquots were then spread on appropriate selective media containing 0.25% sodium citrate.

Transductants were purified by streaking to single cells. The resulting colonies were tested for PI lysogeny by cross-streaking over P1vl and by streaking over AB3064 embedded in soft agar.

**Isolation of a non-inducible λimm434 mutant**

This method was based on that described by Eshima et al. (1972). λimm434 was irradiated with 3000 ergs mm$^{-2}$ and plated with C600 on LTA plates each containing 1μg of mitomycin C. The plates were incubated at 42°C overnight. Under these conditions λimm434 gave clear plaques since lysogens were induced by the mitomycin C. Presumptive ind$^-$ lysogens were picked from turbid centred plaques (which occurred with a frequency of about 10$^{-3}$) and streaked to single cells. Isolated colonies were then re-streaked to single cells and tested for the presence of λimm434 and UV inducibility.

An overnight culture of a non-inducible lysogen was chlorophormed
and the debris removed by centrifugation. This yielded a preparation of phage liberated by spontaneous induction, which was used to lysogenise W3350. This lysogen was also non-inducible by UV showing that the ind" mutation mapped in the phage genome (see Chapter VI).

Synchronous Cultures Derived Using the Membrane Elution Technique

The technique was based on that described by Helmstetter and Cummings (1964).

A colony of either E.coli B/r (λ) or B/r was inoculated into glucose HC medium and grown for 24 hours. The resulting stationary cultures were then diluted into fresh glucose HC medium and shaken in acid cleaned flasks for at least 15 hours at 37°C until they had reached a cell number of between 5 x 10^7 and 10^8 cells/ml. Three litres of B/r grown in this manner was filtered through a 124mm diameter thick pre-filter and a 0.65μm pore 142mm (type DA) Millipore membrane filter (Millipore Filter Corp., Bedford, Mass., U.S.A.) The filtration apparatus consisted of a Millipore 142mm Filter Holder (Cat. No. YY 2214200) and pressure was supplied using a Millipore pressure tank (Cat. No. XX 6700051). The filtrate, collected in sterile flasks, was known as conditioned medium (CM). A non-lysogen was used to acquire the CM in order that it should not contain large quantities of phage liberated by spontaneous induction. After filtration the filter and pre-filter were removed from the filter holder and 5 l. sterile distilled water was forced through the apparatus.
E.coli B/r (λ) was then bound to a membrane by filtering a 500 ml culture, grown in the manner described above, through a 0.22μm pore, 142mm diameter Millipore filter (type GS) under a pressure of 5 lb/sq in. The filter was then removed from the filter holder, together with the support screen (Cat. No. YY 3014254). The filter was inverted on the support screen and they were clamped into the apparatus shown in Fig.2.1. This operation was performed as rapidly as possible. CM was then allowed to drip onto the filter and clip A (Fig. 2.1) was opened briefly to allow the top of the filter to be covered with medium. A flow rate of approximately 60 ml/min through the filter was maintained for 10-15 minutes to wash free unbound cells. The flow rate was then reduced gradually to 20-25 ml/min using clip B (Fig. 2.1) and elution was continued for a total of 80-85 minutes. A sample was collected for 1½-2 minutes in an acid cleaned 150 ml flask, which was then incubated in a shaking water bath at 37°C.

The bottom portion of the apparatus shown in Figure 2.1 was rotated gently for 20-30 minutes before removing the sample to ensure a smooth flow of medium from the filter.

In order to maintain the cells at a constant temperature throughout the experiment (usually 37°C), all manipulations up to and including the collection of the sample were performed in a constant temperature room.

**Rapid Screening for UV and X-ray Sensitivity**

0.01 ml volumes of exponentially growing cells were streaked across LTA plates. To test for UV sensitivity, half the streaks were irradiated with a suitable dose of UV radiation (usually 400 ergs mm⁻²).
To test for X-ray sensitivity, the streaks were exposed for 15 minutes on the top shelf of a Faxitron Model 804 radiation inspection unit at 125 KVP, 6 ma.

An appropriate dose of radiation prevented growth of a UV or X-ray sensitive strain, but had little effect on the growth of resistant strains.

**Preparation of Bacterial Strains by Mating**

Male and female strains were grown to $2 \times 10^8$ cells/ml with bubbling aeration at 37°C in Luria Broth supplemented with thymine where appropriate. 2 ml of each parental strain were mixed in a 150 ml flask and shaken gently at 37°C. The mating was interrupted by diluting a sample of the mating mixture in PB and agitating vigorously for 5 seconds using a blender similar to that described by Low and Wood (1965). Appropriate dilutions were plated on selective agar.

**Isolation of Streptomycin Resistant Mutants**

Spontaneous mutants were isolated by plating about $3 \times 10^9$ cells on NA plates supplemented with 200 μg/ml of streptomycin.

**Determination of Particle Number**

Particle number was estimated by adding a sample of cells to an ice-cold formaldehyde-saline solution. The final concentration of formaldehyde was 1%. Formaldehyde-killed cells were stored for less than 24 hours at 4°C then the particle number was estimated using a Coulter Counter Model B. Corrections were made for the number of particles present in the media.

-33-
Determination of absorbance

Absorbance was measured using a Gilford Micro-Sample Spectrophotometer.

Measurement of DNA Synthesis

Cultures were incubated with either $^{3}$H thymidine or $^{14}$C thymine. Incorporation of label was stopped by adding samples to ice-cold trichloroacetic acid (TCA) containing either unlabelled thymidine or thymine at 100 µg/ml. The final concentration of TCA was 5%.

After storage for at least 40 min at $0^\circ$C the TCA precipitates were collected on 27mm diameter Sartorius membrane filters which had already been washed with 5ml TCA containing either 100 µg/ml thymidine or thymine. The precipitates were washed with 12 x 5ml volumes of boiling water and the filters were dried under an infra-red lamp. They were immersed in scintillation fluid and the radioactivity was counted using a Packard 526 liquid scintillation spectrophotometer.
Figure 2.1 - Diagram of the Apparatus used to obtain Synchronous Cultures by the Membrane Elution Technique

1. Sterile aluminium foil.
2. 200mm Pyrex glass filter funnel.
3. Reservoir of CM.
4. Autoclaveable flexible tubing and connector.
5. Quickfit flask lid (in which the centre socket was replaced by glass tubing and a glass tubing side was fitted).
6. GS Millipore membrane.
7. Teflon coated support screen (Millipore Cat. No. YY 3014254).
8. G clamp.
9. Quickfit flask lid, the top of which was removed and replaced by a 100mm Pyrex glass filter funnel.
10. Rubber tubing.
CHAPTER III

DEVELOPMENT OF AN ADEQUATE TECHNIQUE FOR OBTAINING SYNCHRONY

Before examining whether lysogens of different ages show a variation in their ability to be induced, it was necessary to develop a reliable method for obtaining a population of cells of uniform age.

Many techniques exist for obtaining synchrony of division in bacterial populations (for a review see Helmstetter 1969). Abbo and Pardee (1960) have distinguished between synchrony obtained by exposing a culture to a regime of environmental change resulting in the phasing of the entire culture and yielding synchronised populations of cells and synchrony obtained by selecting cells of the same age out of an exponential culture, yielding synchronous populations. This distinction is to some extent blurred since any selection method must involve temporary environmental change. Nevertheless, in general techniques of the latter type must be regarded as more satisfactory since it is difficult to be certain how extensive the perturbations to cell growth necessarily involved in forcing an asynchronous culture to divide in synchrony will be.

However, the first method used was a synchronising technique and was based on that described by Cutler and Evans (1966). In essence, this is extremely simple consisting of the resuspension of cells grown to stationary phase in fresh medium. The data recorded
in the original report show high quality phasing over several generations, regular doublings in cell number, these doublings being separated by roughly constant intervals of time which are approximately equal to the generation time of the original exponentially growing culture.

The quality of the data of Cutler and Evans (1966) could not be reproduced and the results which were obtained were unsatisfactory. Figure 3.1 shows one of the better results obtained with this technique. In this experiment cells were harvested from the culture 1½ hours after the cells had entered stationary phase. This time is defined as shown in Figure 3.1.

Samples removed at times between 1 and 3 hours after the cells had reached stationary phase, in different experiments, did not give reproducible results in the quality of the synchrony. In general, the times at which synchronised divisions occurred were often not those predicted from the growth rate of the parent culture and apparently synchronised divisions often did not result in a doubling in cell number.

Although a satisfactory explanation of why this technique produces synchrony is lacking, one possibility is that it is due to a bunching effect on the population as it reaches stationary phase (Cutler and Evans 1966). That is, cells approaching stationary phase are growing faster than those already in it and some stage may be reached at which the majority of cells in the population are in a similar physiological condition and thus will divide in synchrony.

-36-
when placed in conditions favouring growth. Since C600 enters its stationary phase rather abruptly (Figure 3.1) the experiment was repeated using P4X which enters its stationary phase more gradually (Figure 3.2) which may favour alignment of the cells. However, no satisfactory synchrony was obtained using this strain as can be seen from Figure 3.2.

Owing to the irreproducibility of the results and the poor synchrony, this technique was abandoned in favour of the membrane elution technique described by HelmsATTER and Cummings (1964) which is one of the best available for obtaining synchronous populations of cells. One of the major limitations of this technique is that it only works with high efficiency with E. coli B/r, although adaptations have been reported which give reasonable results with other strains (Cummings 1970; Shehata and Marr 1970). The method involves the filtering of an exponentially growing population of cells onto a Millipore membrane to which a certain proportion of the population bind. The membrane is then inverted and medium is passed through it so that the bound cells continue to grow on the surface of the membrane until they divide. At division there is a high probability that one of the two daughter cells will be released from the membrane and pass into the eluate. By this technique one can obtain a continuous supply of new born cells, a sample of which can be collected. These cells will then grow through their cell cycle in synchrony.

E. coli B and strains derived from it are generally resistant to λ because they lack a functional malB gene (Chung and Greenberg 1968).
A \textit{mal}$^+$ derivative of B/r was therefore derived by P1 transduction using B251 as a donor. B251 is a \textit{mal}$^+$ strain of \textit{E.coli} B which plates $\lambda$ with high efficiency provided that the phage DNA is not subject to restriction in it (Arber and Dussoix 1962).

\textit{E.coli} B/r \textit{mal}$^+$ ($\lambda$) (referred to below as B/r ($\lambda$)) gave satisfactory synchrony using the membrane elution technique which is described in detail in Chapter II. Figure 3.3 shows synchronous growth of B/r ($\lambda$) in glucose HC medium with a generation time of 39 minutes. The culture shows regular doublings in cell number and the mid-point of the steps is constant and equal to the generation time in exponentially growing cultures. This technique was used to obtain the synchronous populations of cells used in the experiments described in Chapter IV.
Figure 3.1 - Synchronisation of C600 using the Stationary Phase Technique.

An overnight culture of C600 grown in S1 medium at 37°C without aeration was diluted into pre-warmed S2 medium and shaken vigorously at 37°C in a water bath. The absorbance at 450 nm (A_{450}) was measured at intervals. The beginning of stationary phase was arbitrarily defined, as is shown in the diagram, and is indicated by the arrow labelled S. 1\frac{1}{2} hours after the cells had reached stationary phase, at the time indicated by the arrow labelled H, 4ml of the culture was removed and centrifuged. The cells were resuspended in pre-warmed S2 medium at an A_{450} of about 0.2 and incubated with shaking aeration at 37°C. Samples were removed at intervals and the particle number determined.
Figure 3.2 - Attempted Synchronisation of P4X using the Stationary Phase Technique.

The experiment was similar to that described in the legend to Figure 3.1, using strain P4X instead of C600. Two samples were removed at the times indicated by the arrows labelled A and B.
Figure 3.3 - Synchronous growth of \textit{E. coli} B/r (\(\lambda\)) in Glucose HC Medium.

B/r (\(\lambda\)) was filtered onto a Millipore membrane and eluted with conditioned medium as described in Chapter II. After 85 minutes of elution, a sample of the eluate was collected for 2 minutes in a 150ml flask which was then shaken vigorously in a 37\(^\circ\)C water bath. At intervals thereafter samples of the culture were removed and particle number determined.
CHAPTER IV
LAMBDA INDUCTION AND THE BACTERIAL CELL CYCLE:
STUDIES WITH SYNCHRONOUS CULTURES

UV Induction of Synchronously Growing E. coli B/r (λ)

The inducing agent used in these experiments to test the hypothesis that cells at different stages in the cell cycle can be induced with different efficiency was UV irradiation. This was because it induces λ efficiently and its effects on the cell are better understood than those of other inducing agents.

A series of samples of a synchronous population of lysogens, obtained by the membrane elution technique, were exposed to a dose of UV irradiation which would induce only a small fraction of cells in an exponentially growing culture. If the hypothesis outlined above is correct, a peak of inducibility should define the time in the cell cycle when prophage λ is most readily induced.

Induction was measured using an infective centres assay and is expressed as the fraction of lysogenic cells which give rise to infective centres after UV irradiation. E.coli B251 was used as an indicator strain to measure phage production from B/r (λ) because λ formed small, ill-defined plaques on B/r mal\(^+\) transductants. Eighty transductants were tested and were found to plate λ with an efficiency of 20-30\% of that on B251 at 37°C. On B251 at 37°C λ forms large, clearly defined, turbid plaques. The reason for the poor plating ability of λ on B/r mal\(^+\) strains may be due to the reported temperature sensitivity of maltose utilisation and λ sensitivity in E.coli B.
(Ronen and Raanen-Ashkenazi 1971). However, this did not appear to be true of the B251 strain used in these experiments, which seemed to adsorb λ efficiently at 37°C, as judged by plaque morphology.

The inducibility of a synchronous population of B/r (λ) was found to vary with its age as is shown in Figure 4.1. The pattern observed, with a peak of inducibility occurring about 23 minutes before division, proved to be repeatable. The relevant evidence for this, from five independent experiments, is summarised in Table 4.1. The time at which the position of the peak of inducibility was observed was taken as the time at which the maximum fraction of the population was inducible and the time at which cell division occurs was taken as the time at which half of the population had divided.

The number of infective centres formed following the plating of unirradiated samples of synchronous cultures has been subtracted from the data shown in Figure 4.1 (and subsequent experiments) to give a more accurate measure of the level of UV induction. In these experiments, considerably more infective centres were produced by unirradiated samples than could be explained on the basis of spontaneous induction between the time of plating and the addition of streptomycin. This is indicated in Figure 4.2, where the numbers of infective centres are expressed as a fraction of the number of cells present in the culture (percentage infective centres). Most of these infective centres were due to phages present in the sample of eluate which was collected from the membrane for use in these experiments. This was indicated by the observation that the level of spontaneous induction of lysogens centrifuged free of elution medium (0.35%) did not differ
### TABLE 4.1

**UV Induction of a Series of Synchronous Cultures of E. coli B/r (λ)**

<table>
<thead>
<tr>
<th>Generation time (min)</th>
<th>Distance from first peak to subsequent division (min)</th>
<th>Distance from first to second peak (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>20.5</td>
<td>39.0</td>
</tr>
<tr>
<td>39</td>
<td>22.5</td>
<td>37.0</td>
</tr>
<tr>
<td>38</td>
<td>24.5</td>
<td>37.0</td>
</tr>
<tr>
<td>38</td>
<td>26.0</td>
<td>37.0</td>
</tr>
<tr>
<td>38</td>
<td>22.5</td>
<td>38.5</td>
</tr>
</tbody>
</table>

**Average:** 38.2  

The experimental details are those given in the legend to Figure 4.1. The times at which the peaks and division occurred were determined as described in the text.
significantly from the level observed in an asynchronous culture (0.21%). Furthermore, chloroformed samples of the harvested eluate contained quantities of phages sufficient to account for the large numbers of infective centres formed by the unirradiated samples (0.7%).

A final point which may be noted about the data presented in Figure 4.1 is that the average efficiency of induction of the synchronous population of cells is approximately equivalent to the efficiency of induction of the asynchronous parental population of cells at the UV dose used.

**Thermal Derepression of λ in Synchronous Cultures**

The variation observed in the ability to produce infectious phage following UV irradiation of cells of different ages could be due to a variation in the ability of the phage to grow in irradiated cells subsequent to derepression rather than to a variation in the inducibility of the population. To investigate this further, use was made of a lysogen carrying λ cI857 ind" as a prophage. The ind" mutation (Jacob and Campbell 1959) prevents the induction of this prophage by UV irradiation (Figure 4.3). The cI857 mutation (Sussman and Jacob 1962) results in the synthesis of a repressor whose stability depends on the temperature. Thus at 41°C, B/r (λ cI857 ind") becomes rapidly derepressed (Figure 4.4).

To determine whether irradiated cells of different ages differ in their ability to support phage growth, samples were removed at intervals from a synchronous culture of B/r (λ cI857 ind") growing...
at 31°C. The samples were lightly UV irradiated, then briefly incubated at 41°C to irreversibly derepress a small fraction of the population. This experiment is complicated by the fact that, as can be seen from Figure 4.4, this strain is extremely sensitive to thermal derepression so that slight variations in the length of the heat pulse at 41°C and slight fluctuations in the temperature of incubation would be expected to have profound effects on the fraction of cells giving rise to infective centres. This probably accounts for most of the variation observed in the fraction of cells giving rise to infective centres after transient derepression of samples of lysogens of different ages (Figure 4.5).

However, the variation does not form a pattern comparable to that observed following UV irradiation, as can be seen by comparing Figure 4.1 with Figure 4.5. The tendency apparent in Figure 4.5 for the number of cells giving rise to infective centres to increase at division is probably due to the manner in which this fraction was calculated. The number of cells used in the calculation was taken as that at the time of removal of the sample, but the effect of an 85 second heat pulse at 41°C, at a time when many cells are close to division, may mean that following the heat pulse, the number of cells is significantly greater. The increase in the number of cells giving rise to infective centres at division may, therefore, be more apparent than real.

The UV Inducibility of Synchronous Populations of Lysogens

Defective in Excision Repair

It has been shown above that there is a variation in the efficiency with which UV can induce lysogens of different ages (see
Figure 4.1). One could consider two explanations for this type of variation. The first explanation is that a lysogen of any given age has a fixed probability of being induced by UV. The second explanation is that induction depends upon whether some component which varies through the cell cycle can accumulate before the cell recovers from the effects of irradiation. This is illustrated by the following example. If induction is caused by the accumulation of some component involved in the initiation of rounds of DNA replication, then the probability that a lysogen will become induced depends upon whether DNA synthesis is inhibited long enough for this component to accumulate above the point at which it would normally be involved in initiation. Thus, the longer DNA synthesis is inhibited, the greater the probability that cells containing relatively small amounts of the component at the time of irradiation will become induced. A consequence of the second explanation is that the variation in the efficiency of induction should be reduced if the effects of UV on the lysogen can be made more severe or long lasting by, for example, reducing its DNA repair potential.

In an attempt to investigate this problem further, *E. coli* WP2 hcr, which is defective in excision repair was used. Since, as discussed in Chapter I, UV induction requires the continued presence of excisable UV photoproducts, inactivation of the excision repair system should greatly reduce the rate at which the lesions are removed from the DNA. *E. coli* WP2 hcr was isolated from a tryptophan requiring auxotroph of *E. coli* B/r (Hill 1965). Hill (1970) has mapped the hcr mutation in this strain close to malB using a mating system. This map position was confirmed in the present study using P1 transduction.
Of 103 mal\(^+\) transductants, 66 were found to be UV resistant when tested by a streak test (see Chapter I), only 37 retaining the UV sensitivity of \textit{E.coli} WP2 \textit{hcr}. The \textit{uvrA} and \textit{malB} mutations are co-transducible with high frequency (70\%) in \textit{E.coli} K12 (Schwartz 1966) and it is likely that the \textit{hcr} mutation in \textit{E.coli} WP2 is equivalent to an \textit{E.coli} K12 \textit{uvrA} mutation as suggested by Hill (1970).

A strange property of WP2 is that in the absence of 0.05\% sodium citrate it grows poorly in the glucose HC medium used for \textit{E.coli} B/r. The generation time is about 75 minutes compared to 42 minutes if sodium citrate is present. The reason for this is not understood but sodium citrate was routinely added to media in the experiments described below. This observation suggests that \textit{E.coli} WP2 may differ genetically from the \textit{E.coli} B/r strain used in this work at loci other than \textit{hcr} and \textit{trp}.

UV induction curves for asynchronous cultures of \textit{E.coli} B/r (\(\lambda\)) and WP2 \textit{hcr} (\(\lambda\)) are shown in Figure 4.6. The excision defective mutant is more efficiently induced by UV at small UV doses for reasons discussed in Chapter I. The efficiency with which UV induces WP2 \textit{hcr} (\(\lambda\)) cells of different ages is shown in Figure 4.7 and can be compared with the data obtained using an excision proficient transductant of this strain (Figure 4.8).

The key points to be noted in Figure 4.7 are first, there is comparatively little variation in the efficiency of induction through the cell cycle and second, that there is no peak of inducibility corresponding to that observed with the excision proficient strain. Although there appears to be some variation in inducibility, the
peak occurs slightly earlier in the cell cycle than that observed using a repair proficient mutant.

This pattern of residual variation could be due to the action of another repair system. Alternatively, cells which require extensive growth after irradiation before derepression may lose the capacity to support phage growth.

The results of this experiment seem to rule out the hypothesis that the variation in inducibility through the cell cycle is solely a function of the age of the cell at the time of irradiation. This conclusion is supported by the observation that the variation in inducibility of lysogens is reduced at higher UV doses as can be seen by comparing Figure 4.1 with Figure 4.9.

**The Effect of Growth Rate on the Position of the Peak of UV Inducibility**

Having observed a variation in the inducibility of \( \lambda \) lysogens during their division cycle, an attempt was made to correlate the age of optimal induction with some stage in the DNA replication cycle.

According to the model of Cooper and Helmstetter (1968), for *E.coli* B/r growing with generation times between 20 and 60 minutes, the completion of a round of DNA replication occurs \( D \) minutes before cell division, where \( D \) is constant and equal to about 22 minutes. The initiation of a round of DNA replication occurs \( C \) minutes before it is completed, where \( C \) is also constant and is equal to about 41 minutes. These measurements were made using cells growing on the surface of a membrane where their generation time is some 10% less than in equivalent batch cultures. The values of \( C \) and \( D \) for experiments described in this Chapter may, therefore, be 10% higher than
those indicated, i.e. 45 and 24 minutes respectively.

A consequence of this model is that for cells growing with generation times of less than 60 minutes, the completion of a round of DNA replication always occurs at a constant time before division, whereas the time at which initiation occurs will vary in its timing relative to cell division according to the growth rate.

The variation in inducibility through the cell cycle in synchronous populations of cells growing at four different growth rates is shown in Figures 4.10 and 4.11. In Figure 4.11 the variation in inducibility is compared with the percentage of cells dividing per 5 minutes through the cell cycle, instead of cell number as in Figure 4.10.

It can be seen that the peak of inducibility occurs 21, 23, 24 and 30 minutes before division at growth rates of 34, 38, 58 and 80 minutes respectively. Thus, with the possible exception of the slowest growth rate, using sodium succinate as a carbon source, the cells appear to be most readily inducible close to the time one would expect a round of DNA replication to be terminating. In two experiments not shown, using sodium succinate as the carbon source, the peaks of UV inducibility occurred 25 and 26 minutes before division, but the quality of the synchrony in these experiments was worse than that shown in Figure 4.10.

The times at which initiation of rounds of DNA replication would be most likely to occur at the growth rates used, according to the model of Cooper and Helmsatter (1968) are shown in Figure 4.12,
together with the times at which the peaks of UV inducibility were observed. The time at which rounds of replication are initiated at growth rates greater than 60 minutes is somewhat controversial. According to Cooper and Helmstetter (1968), where the generation time \( \tau \) is greater than 60 minutes, \( C \) is approximately equal to \( \frac{2}{3} \tau \) and \( D \) is equal to about \( \frac{1}{3} \tau \). In other words, initiation occurs at birth and DNA replication occupies the first two thirds of the division cycle. This view has been challenged by Kubitschek and Freedman (1971) who claim that the values of \( C \) and \( D \) remain constant and independent of the growth rate even at generation times considerably longer than one hour. Data described in the next section tend to support the latter view under the conditions used in these experiments.

One final point which may be noted about the data presented in Figures 4.10 and 4.11 is that, irrespective of the growth rate, cells are least inducible at the time when the largest fraction of the population is dividing.

### Measurement of DNA Synthesis in Synchronous Cultures

The interpretation of the results presented in the previous section depends upon the assumption that the model of Cooper and Helmstetter (1968) validly describes growth under the experimental conditions used.

An attempt was made to measure the rate of DNA synthesis through the cell cycle in cells growing in sodium succinate. In order to do this, samples were removed at intervals through the cell cycle and pulse labelled with \(^3\text{H}\) thymidine. Initiation of a round of DNA replication should result in a sharp increase in the amount of \(^3\text{H}\)
thymidine incorporated into DNA in a pulse.

One of the difficulties with this experiment is that because of the technique used to acquire the synchronous cultures, the concentration of cells available is rather small, rarely exceeding $2 \times 10^6$ cells/ml. Thus, in order to ensure significant uptake of radioactively labelled DNA precursor, it is necessary to pulse label for 5 minutes even using a high specific activity. This is shown by the uptake curve in Figure 4.13.

The results of pulse labelling two synchronous cultures growing in sodium succinate are shown in Figure 4.14. It can be seen that there is an increase in the rate of DNA synthesis early in the first division cycle, which levels off and then starts to increase again as the cells enter the second division cycle. This can be interpreted to mean that cells initiate a round of DNA replication early in the cell cycle, before the age at which they are maximally inducible. However, there is no clear indication of a decrease in the amount of $^3$H thymidine incorporated into DNA late in the division cycle, when termination should occur. This is probably due to the fact that the quality of the synchrony obtained with cells growing in sodium succinate was consistently poor and that at all growth rates the quality of the synchrony of the population deteriorates rapidly during incubation following collection of the sample of eluate from the membrane.

**Discussion**

The data presented in this Chapter shows that there is a variation in the efficiency with which lysogens can be induced during
the cell cycle. It has been shown that lysogens growing at different growth rates are optimally inducible when UV irradiated between 21 and 30 minutes before cell division and that they are least readily inducible at division. These experiments render it highly unlikely that induction is caused by the accumulation of some component involved in the initiation of rounds of DNA replication since, for a range of growth rates, the position of the peak of inducibility does not correlate with the various times when it is thought that rounds of replication are initiated.

It is interesting that, as noted in Chapter I, there is a 20 to 30 minute lag between exposure of lysogens to an inducing treatment and the derepression of the prophage. It may be significant that a similar period of time elapses between the age of optimal induction of a synchronous population of lysogens and the age at which they would normally have divided and between the time of irradiation of a population of *E. coli* B/r and the time at which division of the population is observed to be inhibited (Clark 1968a; Helmsatter and Pierucci 1968). These observations seem to support the hypothesis that induction and the inhibition of cell division are related.

However, Clark (1968a) and Helmsatter and Pierucci (1968) have interpreted their data to mean that completion of a round of replication is essential for cell division to occur and that following completion a constant period of about 20 minutes elapses before cell division takes place. Thus if DNA synthesis is briefly inhibited at any cell age both the time of termination and the subsequent cell division should be delayed. On this hypothesis, if induction and
the inhibition of cell division are related it is surprising that any variation in inducibility can be observed in synchronous populations of glucose-grown cells, where DNA replication is continuous through the cell cycle, since a brief period of inhibition of DNA synthesis at any given age should ultimately lead to the inhibition of cell division. In the case of cells less than 20 minutes before division, it would be the division after the one they are about to undergo which would be inhibited.

Recent work suggests that delaying the time at which a round of DNA replication is completed need not necessarily affect the timing of cell division. First, Jones and Donachie (1973) used cells which had completed rounds of DNA replication in the absence of protein synthesis then allowed protein synthesis to resume whilst blocking DNA replication by thymine starvation. Thymine was added back to the growth medium after all of the cells had reached the mass necessary for rounds of replication to be initiated and a synchronised wave of DNA replication resulted. Under these conditions, the interval between termination and division can be reduced to as little as 5 minutes, suggesting that much of the 20 minutes normally observed between the completion of a round of DNA replication and cell division is required for events which do not require termination to occur but which can take place in parallel with rounds of DNA replication. Second, if the rate at which replication forks traverse the chromosome is decreased by substrate limitation (Pritchard and Zaritsky 1970) then the time between completion of a round of DNA replication and the subsequent cell division is also decreased (Meacock and Pritchard,
unpublished data).

Although these results suggest that transitory inhibition of
DNA synthesis following the irradiation of cells need not necessarily
effect the timing of cell division, a difficulty remains in
reconciling the data on the variation in inducibility through the
cell cycle with the hypothesis that induction and the inhibition of
cell division are related. If the inhibition of cell division is solely
dependent on the inhibition of DNA synthesis then irradiation of cells
at any given age in the cell cycle should ultimately lead to the same
inhibitory effect on cell division provided that there is a similar
inhibitory effect on DNA synthesis at all ages.

The hypothesis that induction and the inhibition of cell division
are related can only be sustained therefore if it is assumed that the
inhibitory effect which UV has on cell division is partly or wholly
independent of the inhibitory effect which it has on DNA synthesis.
Evidence supporting this assumption will be presented in the next
Chapter.

The experiments reported in this Chapter have so far been
considered in terms of the hypotheses which they were primarily
designed to investigate. They can now be considered in terms of the
other hypothesis which was outlined in Chapter I as a possible explana-
tion for the mechanism of prophage induction. This hypothesis is that
induction is caused by the action of some repair system. Such a
hypothesis could be used to explain the data presented in this
Chapter if it is assumed that there is a variation in repair enzyme
activity through the cell cycle. Clark (1968b) has suggested that
recA mediated repair of ionising radiation damage may be more efficient in the middle of the cell cycle in glucose-grown cells than in newborn or old cells. However, the data presented in support of this argument are not good enough to draw firm conclusions.

Possible reasons for a fluctuation in repair activity could be that there is a variation in the relative activity of different repair enzymes or a variation in the amount of irradiated DNA per cell or a variation in the susceptibility of the irradiated DNA to repair enzymes during the cell cycle.

A variation in the relative activity of different repair enzymes might be based, for example, on changes in the size of nucleoside triphosphate pools which are known to occur during the cell cycle of E. coli B/r (Huzyk and Clark 1971). If nucleoside triphosphates function as co-factors in some repair reactions, a change in their pool size might preferentially favour the action of one repair system in competition for the available UV photoproducts at a certain stage in the cell cycle.

The amount of DNA per cell compartment also fluctuates during the cell cycle and at growth rates where DNA synthesis is continuous through the cell cycle it reaches a maximum a few minutes before physical separation of the daughter cells (Clark 1968a). Since there is a variation in the amount of DNA per cell compartment, there should also be a variation in the number of UV photoproducts introduced per cell for any given UV dose and consequently a variation in the amount of substrate available for repair enzymes to act upon. However, it is unlikely that this could account for the observed variation in inducibility through the cell cycle. It would predict a similar
pattern in the variation in inducibility of both excision proficient and excision defective lysogens since the DNA content of the latter should fluctuate in the same way as the former.

The possibility that there is a variation in the susceptibility of irradiated DNA to repair at different stages in the cell cycle could be consistent with the data reported here. The activity or relative activity of some repair system involved in induction could be favoured by the conformation of the chromosome at some stage close to the completion of a round of DNA replication.

To summarise, the data presented in this Chapter are inconsistent with the hypothesis that induction is caused by the accumulation of some component involved in the initiation of rounds of DNA replication. They could be consistent with the hypothesis that induction is related to the inhibition of cell division and they could also be consistent with the idea that induction is related to the activity of a repair system if it is assumed that the relative activity of the repair system involved in induction varies through the cell cycle.
Figure 4.1 - UV induction of E. coli B/r (λ) growing synchronously in glucose HC

Samples were removed at intervals from the synchronous culture, UV irradiated with 12.5 ergs mm\(^{-2}\) and plated with E. coli B251 Sm\(^R\) on LTA plates. Streptomycin was added after 2½ hr incubation at 37°C as described in Chapter II. Samples were also removed for determination of particle number as described in Chapter II.

The dotted line represents the level of induction of an asynchronous culture irradiated with 12.5 ergs mm\(^{-2}\).
Figure 4.2 - Infective centres present in unirradiated samples of synchronous cultures of E.coli B/r (λ)

The experiment was similar to that described in the legend to Figure 4.1 except that the samples were not irradiated. The level of spontaneous induction of the asynchronous culture from which the synchronous culture was derived is shown by the dotted line.
**Figure 4.3** - UV induction curves of *E. coli B/r (λ)* and *E. coli B/r (λ cI857 ind⁻)*

*E. coli B/r (λ)* and *E. coli B/r (λ cI857 ind⁻)* were grown in glucose HC at 31°C to an absorbance at 450 nm of 0.3. Samples were removed, washed, resuspended in PB and irradiated. Appropriate dilutions were plated with *E. coli B251 Sm⁸* on LTA plates which were incubated for 2½ hr at 30°C then overlaid with streptomycin (see Chapter II).
Fig. 4.4 - Irreversible de-repression of \textit{E. coli} B/r (\textit{\lambda} cI857 \textit{ind}^-)

by growth at 41°C

An exponentially growing culture of \textit{E. coli} B/r (\textit{\lambda} cI857 \textit{ind}^-) was centrifuged, the cells resuspended in pre-warmed conditional medium and incubation continued at 31°C. Samples were removed at intervals, diluted into medium warmed to 31°C, irradiated with 12.5 ergs mm^-2 and transferred to a flask in a shaking water bath at 41°C. After incubation at 41°C for varying lengths of time, the flasks were transferred to an ice-water bath, shaken for 2 minutes, then the contents were diluted and plated with \textit{E. coli} B251 Sm^R on LTA plates. After 3 hours of incubation at 30°C, streptomycin was added to the plates (see Chapter II). Particle number was determined as described in Chapter II.
% Infective centres

Time at 41°C (min)
Figure 4.5 - The effect on a heat pulse at $41^\circ$C on the production of infective centres by a synchronous culture of E. coli B/r ($\lambda$ cI857 ind$^+$) growing at $31^\circ$C.

Samples were removed at intervals from a synchronous culture of E. coli B/r ($\lambda$ cI857 ind$^+$) growing at $31^\circ$C, irradiated with 12.5 ergs mm$^{-2}$, incubated at $41^\circ$C for 85 seconds and plated out for infective centres. The details are the same as those given in the legend to Figure 4.4. A second experiment (indicated by the dotted line) differed from the first in that samples were diluted into medium at room temperature prior to irradiation. This accounts for the lower proportion of cells forming infective centres in this experiment.
Figure 4.6 - UV Induction Curves for *E. coli* WP2 hcr (λ) and *E. coli* B/r (λ)

*E. coli* WP2 hcr (λ) in HCTC and *E. coli* B/r (λ) in glucose HC were grown to an absorbance at 450nm of 0.3. The experiment was similar to that described in the legend to Figure 4.3 except that incubation was at 37°C.
The experiment was similar to that described in the legend to Figure 4.1 except that samples were irradiated with 6.25 ergs mm$^{-2}$. The open circles represent the average data for percentage induction from three separate experiments. The arrows represent the positions of the peaks of induction in an excision proficient strain (see Figure 4.8).
Figure 4.8 - UV Induction of *E. coli* WP2 hcr<sup>+</sup> (λ) Growing Synchronously in HCTC

The experiment was similar to that described in the legend to Figure 4.1
Figure 4.9 - UV Induction of E.coli B/r (λ) Growing Synchronously in Glucose HC: the Effect of Increased UV Dose

The experiment was similar to that described in the legend to Figure 4.1 except that the samples were irradiated with 25 ergs mm⁻². The dotted line represents the level of induction at this UV dose of the asynchronous culture from which the synchronous culture was derived.
Figure 4.10  -  UV Induction of E.coli B/r (λ) Growing Synchronously 

                        at Various Growth Rates I

The experiment was similar to that described in the legend to Figure 4.1 except that the cells were grown (a) in glucose HC supplemented with cysteine and methionine, (b) in HC minimal salts containing proline and alanine as carbon sources, (c) in HC minimal salts containing sodium succinate as a carbon source.
Figure 4.11 - UV Induction of E.coli B/r (λ) Growing Synchronously at Various Growth Rates II

Data taken from Figure 4.1 and 4.10 have been replotted on a linear scale. The change in the inducibility of the population with time is compared with the fraction of cells dividing in 5 minute intervals, which is plotted as a histogram.

Growth media
(a) Glucose HC
(b) Glucose HC supplemented with methionine and cysteine
(c) HC minimal salts containing proline and alanine as carbon sources
(d) HC minimal salts containing sodium succinate as a carbon source
Figure 4.12 - Predicted Times of Initiation of Rounds of DNA Replication in Cells Growing with Different Growth Rates

The shaded areas represent the times at which initiation should occur according to the models of Cooper and Helmbstetter (1968) and Kubitschek and Freedman (1971). For growth rates less than 60 minutes, C is taken as 41 to 45 minutes, D as 22 to 24 minutes. The arrows show the observed positions of peaks of inducibility.
Time (min)

\[ \tau = 34 \]

\[ \tau = 38 \]

\[ \tau = 58 \]

\[ \tau = 80 \]
Figure 4.13 - Uptake of $^3$H thymidine by an Exponentially Growing Culture of *E. coli* B/r (λ)

$^3$H thymidine was added to a culture containing about $10^6$ cells/ml of *E. coli* B/r (λ) growing exponentially in sodium succinate at 37°C. The specific activity of $^3$H thymidine was 25 µCi/µg and the concentration of thymidine was 1 µg/ml. Samples were removed from the culture at intervals and the incorporation of label stopped as described in Chapter II. The method of washing and counting the samples is also described in Chapter II.

cpm/ml = counts per minute incorporated into acid insoluble material per ml of culture.
Figure 4.14 - Pulse Labelling of *E. coli* B/r (λ) Growing Synchronously in Sodium Succinate HC

Samples were removed at intervals from a synchronous culture of *E. coli* B/r (λ) growing in sodium succinate HC at 37°C. The samples were incubated with ^3^H thymidine for 5 minutes at 37°C, then incorporation of label was stopped and the samples washed and counted as described in the legend to Figure 4.13 and Chapter II.
Expt. 1
Expt. 2
CHAPTER V

LAMBDA INDUCTION AND THE BACTERIAL CELL CYCLE:
STUDIES WITH ASYNCHRONOUS CULTURES

The Effect of Growth Rate on the UV Inducibility of
Lysogenic Cells

The experiments described in this Chapter were designed to
confirm and extend the conclusions reached in Chapter IV that there
is a particular stage in the cell cycle at which λ is most readily
induced. This conclusion was based primarily on observations on
the variation of UV inducibility through the cell cycle of a series
of synchronous cultures growing with different generation times.

When UV induction curves for asynchronous cultures growing
with different generation times were compared, the results shown in
Figure 5.1 were obtained. These curves show the fractions of cells
growing with different generation times which give rise to infective
centres after exposure to a variety of UV doses.

It can be seen from Figure 5.1 that at all growth rates, the
largest proportion of the population is induced at a UV dose of
approximately 150 ergs mm\(^{-2}\). However, the size of the maximum
inducible fraction of the population is proportional to the rate at
which the culture was growing before UV irradiation.

This phenomenon can be explained in two ways. First, the
faster cells grow, the more of a factor required for induction they
might contain. In other words, the inducibility of each cell in the
population varies according to the growth rate. Second, the phenomenon
could be explained if there is a particular stage in the cell cycle at which $\lambda$ is most readily induced. According to the latter hypothesis, a larger proportion of fast growing cells are inducible because the faster the growth rate, the larger the proportion of the population which will be near to or of the age of optimal induction.

Examination of Figure 5.1 reveals that at low UV doses, the difference between the fraction of cells induced at the various growth rates is much smaller than at higher UV doses. This would not be expected on the basis of the first hypothesis which would suggest that there should be fewer cells induced at slow growth rates irrespective of the UV dose. However, the second hypothesis predicts that there will be a small fraction of the population close to or at the age of optimal induction at any growth rate, and that the differences in the inducibility of cells growing with different generation times are likely to become much more apparent at higher UV doses.

The Effect of a Shift-up on the Maximum Fraction of Inducible Cells in a Population

Although the data presented in Figure 5.1 are consistent with the hypothesis that in exponentially growing asynchronous cultures the inducibility of a lysogen depends on its age at the time of UV irradiation, no conclusions can be drawn from them as to which cell age provides the most favourable conditions for induction.

In an attempt to analyse the problem further, a lysogenic population of cells was shifted from a medium in which proline and alanine were the carbon sources and in which a maximum of about 20% of the population was UV inducible, to a glucose HC medium supplemented
with casamino acids and tryptophan in which a maximum of about 60% of the population was inducible. The kinetics of transition to the maximal level of induction characteristic of the new medium were then studied.

As discussed in Chapter IV, the bacterial life cycle can be considered in terms of a constant period of time, C, between the initiation of a round of DNA replication and its completion, and another constant period of time, D, between the completion of a round of DNA replication and cell division. In other words, according to this model, the frequency with which cells are completing rounds of DNA replication depends upon the frequency with which they were initiating rounds of replication C minutes earlier, and the frequency with which cells are dividing depends upon the frequency with which they were initiating rounds of replication C+D minutes earlier.

Consequently, when cells are shifted from a relatively poor to a relatively rich medium, although the rate of mass increase accelerates within a few minutes, the cells continue to complete rounds of replication at the pre-shift rate for about C minutes after the shift, and to divide at the pre-shift rate for about C+D minutes (65 - 70 minutes) after the shift, a phenomenon known as rate maintenance(Kjeldgaard et al.1958; Cooper 1969). This phenomenon is clearly illustrated in the data presented in Figure 5.2, where cell division continues at the old rate for 60-70 minutes after a shift-up.

Since initiation of rounds of DNA replication occurs at a constant mass per chromosome origin (Donachie 1968), the frequency with which rounds of replication are initiated depends upon the rate of mass increase. The latter accelerates almost immediately after a shift-up.
although in the experiment described here it takes about 10 minutes for the definitive post-shift rate to be reached (Figure 5.2). Thus the frequency of initiation should also increase rapidly after a shift, and should be very close to its new rate after one mass doubling in the new medium.

These observations enable some predictions to be made about the kinetics of transition to the maximal level of induction characteristic of the new medium after a shift-up. If, for reasons discussed in Chapters I and IV, cells about to initiate a round of DNA replication are more readily inducible than cells of other ages, the level of induction should rise rapidly after the shift and should be close to the level characteristic of the new medium after one mass doubling.

If cells close to the completion of a round of DNA replication are most readily inducible, one would expect the level of induction to be maintained at the pre-shift level for C minutes after the shift then rise rapidly to the new maximal level of induction.

The observed result, shown in Figure 5.2, corresponds to neither of these two predictions. The level of induction increases rapidly after the shift, as would be expected if induction were linked to the accumulation of some component involved in the initiation of rounds of DNA replication, but it does not reach its definitive post-shift level for about 75 minutes after the shift, which is about the time at which cells should be terminating rounds of DNA replication at the new rate.

This result seems to exclude the possibility that it is the stage of the DNA replication cycle which is the sole determinant of whether or not induction occurs efficiently.
UV Induction of a dnaA (λ) Lysogen

The possibility that UV induction of prophage λ and the inhibition of cell division following treatment with inducing agents may have common stages has been considered in Chapters I and IV. It is interesting to examine this hypothesis in the light of the experiments of Monk and Gross (1971), discussed in Chapter I, which show that in a dnaA mutant, which is temperature sensitive for the initiation of rounds of DNA replication, a prophage can be induced by UV under conditions where DNA synthesis has ceased.

An interesting property of many mutants which have been shown to be defective in the initiation of rounds of DNA replication is that they continue to divide after DNA synthesis has ceased at the non-permissive temperature, giving rise to anucleate cells (Hirota et al. 1968). Mutants of Salmonella typhimurium have also been reported which are temperature sensitive for the initiation of rounds of DNA replication (Spratt and Rowbury 1970) and in which cell division continues after DNA synthesis has ceased at the non-permissive temperature (Spratt and Rowbury 1971). It appears, therefore, that although DNA synthesis is blocked in dnaA mutants, at least some cells continue to undergo what could be described as a division cycle.

In view of the hypothesis that the inhibition of cell division and prophage induction may be linked in some way, it is of interest to know of the effect which UV irradiation might have on the residual cell division occurring in dnaA mutants after DNA synthesis has ceased. Hirota et al. (1968) have reported that the production of anucleate cells...
by dnaA mutants can be considerably reduced by treatment with nalidixic acid or thymine starvation, and Shannon et al. (1972) have also reported that nalidixic acid has an inhibitory effect on cell division in a mutant of \textit{S. typhimurium} temperature sensitive for the initiation of rounds of DNA replication, after it has stopped making DNA at the non-permissive temperature. Furthermore, Inouye (1969) has described a temperature sensitive mutant, of the dnaB type, which stops making DNA immediately at 41\textdegree{}C, but which continues to divide producing anucleate cells. He has reported that nalidixic acid but not thymidine starvation can stop division under these conditions. Several published reports suggest therefore that nalidixic acid may have an inhibitory action on cell division which is independent of its inhibitory effect on DNA synthesis. It is interesting to speculate whether UV irradiation might not have a similar effect on residual cell division in a dnaA mutant under conditions where DNA synthesis has ceased.

Figures 5.3a and 5.3b show the result of an experiment designed to show that MC14 (\textlambda{}), a lysogen of the dnaA strain used in the experiments described below is not induced spontaneously at 42\textdegree{}C, but can be induced by UV irradiation as is shown by the stimulation of net DNA synthesis (figure 5.3a) and infectious phage production (figure 5.3b) when irradiated after 75 minutes at 42\textdegree{}C. These results are in good agreement with those obtained by Monk and Gross (1971) with dna-508 (\textlambda{}).

Figures 5.4a and 5.4b show the effect of UV on cell number, absorbance at 450 nm and net DNA synthesis in a culture of MC14 after incubation at 42\textdegree{}C for 75 minutes. These results show that there is
a decrease in the rate of increase in cell number following UV irradiation, but no detectable effect on mass increase (Figure 5.4a) as measured by absorbance at 450 nm, implying that this is the result of an inhibition of cell division rather than an effect on cell growth. There is also no significant net DNA synthesis with or without UV irradiation (Figure 5.4b) during the interval of time studied.

From a measurement of the efficiency of counting (74%) in the experiment shown in Figure 5.3a and from a knowledge of the concentration of thymine (2.5 μg/ml) and the counts incorporated into acid-insoluble-material following UV irradiation of the lysogen (1170 cpm/ml) the DNA synthesised per ml can be calculated in terms of λ genome equivalents. The calculated value \(2 \times 10^9\) λ genome equivalents per ml is far in excess of the \(10^7\) infectious phage particles produced per ml (Figure 5.3b). This might be because the cells are so physiologically disturbed that they are unable to package the phage DNA efficiently after prolonged incubation at 42°C. However, it is interesting to speculate whether the induced phage is able to promote host DNA synthesis by some means. Freifelder et al. (1973) have shown that following thermal derepression of a prophage, excision from the host chromosome usually occurs later than the initiation of DNA replication and that the DNA growing points of the prophage leave the prophage and enter bacterial DNA. In a dnaA mutant it is interesting to speculate whether the host replication machinery could complete rounds of replication initiated from within the prophage. This could account for much of the DNA synthesised which is not incorporated into mature phage particles.
Without a knowledge of the burst size of \( \lambda \) in a \textit{dnaA} mutant after prolonged incubation at 42\(^\circ\)C it is difficult to estimate the efficiency of induction in this system. However, the observation that the number of infectious phage particles released per ml after irradiation is equal to the number of lysogens per ml, suggests that it may be rather low.

**Discussion**

The results shown in Figure 5.1 are consistent with the hypothesis that there is a variation in the inducibility of populations of exponentially growing cells. Attempts to determine indirectly the age at which cells are most readily inducible by means of a nutritional shift-up suggested that this age was not determined solely by the stage of the DNA replication cycle. This argues against the hypothesis, discussed in Chapter IV, that cells close to the completion of a round of DNA replication are most readily inducible because the conformation of the chromosome preferentially favours the activity of a repair system involved in induction. However, the limiting factor in induction may be different under conditions of a shift-up than is the case for lysogens growing in synchronous cultures. In the former case, the sudden increase in the amount of DNA per cell may lead to an increase in the inducibility of the population of lysogens although it is not the limiting factor in determining the optimum inducibility of synchronous populations of lysogens, as was discussed in Chapter IV.

The data on the inducibility of lysogens growing at various growth rates and the effect of a shift-up on the inducibility of lysogens are difficult to interpret. In particular, the effect of a shift-up may produce many temporary perturbations to cell growth which could
profoundly affect the inducibility of the population. For this reason, one can draw no firm conclusions from these experiments.

The second series of experiments described in this Chapter were designed to investigate the possible relationship between prophage induction and the inhibition of cell division. They provide no information on the possible role of repair in induction. These experiments demonstrate that cell division can be inhibited by UV irradiation under conditions where there is little, if any, net DNA synthesis but where cells retain the potentiality for prophage induction. Both the inhibition of cell division of the non-lysogen and the stimulation of net DNA synthesis in the lysogen begin about 15 minutes after irradiation at 42°C.

It is interesting to speculate on the mechanism by which UV might inhibit cell division under these conditions. One possibility is that UV temporarily inactivates one or more genes which code for functions involved in division. This seems rather unlikely, however, unless UV has some specific effect on such genes since irradiation has no detectable effect on mass increase. Another possibility is that UV might act not on DNA but directly to inhibit septum formation, although this also seems rather unlikely. This might be investigated further by determining whether photoreactivation immediately after irradiation restores the ability of the cells to divide.

Electron microscopic studies suggest that following light UV irradiation of bacterial cells, the morphology of the bacterial nucleus changes (Kellenberger 1960) the shape becomes irregular and protrusions are formed such that the nucleus comes to fill almost the entire cell. It is interesting to speculate whether this observation might provide
the basis for an explanation of the inhibition of cell division following UV irradiation. For example, the irradiated DNA might interact with the host membrane, altering the pattern of membrane synthesis and thus affecting septum formation. Although such an explanation can only be regarded as highly speculative, it is interesting to consider it in the light of the observation that several mutants deficient in their repair capacity do not stop dividing under conditions which prevent division in repair proficient cells (see Chapter I). Repair enzymes might be involved in a DNA-membrane interaction either because they further alter the structure of the chromosome by interacting with the UV photoproducts or because they could function to attach the DNA to the membrane as a prerequisite for efficient repair.

Despite the observation that dnaA mutants continue to divide after DNA synthesis has ceased, many of the cells become elongated (Hirot a et al. 1968) suggesting that division is rather inefficient under these conditions. If the hypothesis that prophage induction and the inhibition of cell division is to be maintained, therefore, one of two assumptions must be made. First, it could be assumed that the elongation of dnaA mutants following incubation at 42°C and the inhibition of cell division following UV irradiation result from a fundamentally different cause. Second, it could be assumed that the population is continuing to divide with sufficient frequency to prevent the accumulation of some component involved in induction unless division is further inhibited as a result of UV irradiation.
Figure 5.1 - UV Induction Curves of *E.coli* B/r (λ) grown in a Series of Different Media

The media used in these experiments were: nutrient broth (21); glucose, casamino acids, tryptophan (28); glucose, methionine, histidine (34); proline, alanine (58); sodium succinate (84). The figures in brackets represent the generation times in each of these media. *E.coli* B/r (λ) was grown exponentially in each of the above media to an absorbance at 450 nm of 0.3. Portions of the cultures were removed, washed and resuspended in PB. Following irradiation with an appropriate dose of UV the samples were plated with B251 SmR indicator cells on LTA plates. Streptomycin was added after 2½ hours incubation at 37°C (see Chapter II), this period of incubation being sufficient for maximum expression of induction at all growth rates.
Figure 5.2 - The Effect of a Nutritional Shift-up on the Inducibility of a Population of \( \textit{E. coli} \) B/r (\( \lambda \))

An exponentially growing culture of \( \textit{E. coli} \) B/r (\( \lambda \)) in proline-alanine HC medium was grown at 37\(^{\circ}\)C to an absorbance at 450 nm (\( A_{450} \)) of 0.04, then diluted twofold at the time indicated by the arrow, into fresh HC medium prewarmed to 37\(^{\circ}\)C and supplemented with glucose, casamino acids and tryptophan. Incubation was continued for a further 2 hours. Samples were removed at intervals during the experiment to measure absorbance at 450nm, particle number and inducibility. To determine the inducibility of the population, a sample of the culture was centrifuged, the supernatant discarded and surplus moisture removed from the tube using a sterile cotton bud. The cells were resuspended in PB, a portion removed for determination of particle number and the remainder irradiated with 150 ergs \( \text{mm}^{-2} \). The irradiated cells were plated with \( \textit{E. coli} \) B251 \( \text{Sm}^R \) on LTA plates and streptomycin added after 2\( \frac{1}{2} \) hours incubation at 37\(^{\circ}\)C as described in Chapter II.
Figure 5.3a and b - The Effect of UV Irradiation on DNA Synthesis and Phage Production in MC14 (λ)

MC14 (λ) was grown at 30°C in C medium supplemented with 200 μg/ml deoxyguanosine (Zaritsky and Pritchard 1971) and 14C thymine at a specific activity of 0.05 μCi/μg, the concentration of thymine being 2.5 μg/ml. At an absorbance at 450 nm of 0.2, a portion of the culture was diluted into fresh medium pre-warmed to 42°C and incubation was continued at 42°C. After 75 minutes at this temperature, half of the culture was removed, irradiated with 300 ergs mm⁻² and returned to a fresh flask. Measurements of DNA synthesis were made by adding samples of the culture to ice-cold TCA, and washing and counting the samples as described in Chapter II. Samples were also removed at intervals, chloroformed and plated with C600 on LTA plates to measure the numbers of infective phage produced.
Unirradiated
A  Irradiated

Shift to 4.2°C
UV irradiate

■ Unirradiated
▲ Irradiated

cpm/ml x 10⁻³

Time min

0 40 80 120 160 200

-40 0 40 80 120 160 200
The graph shows the change in infective centres/ml over time at 42°C. The x-axis represents time in minutes, ranging from 70 to 190, and the y-axis represents infective centres/ml, ranging from $10^4$ to $10^7$. Two sets of data points are shown:

- **Unirradiated** represented by filled squares.
- **Irradiated** represented by filled triangles.

The graph demonstrates a significant increase in infective centres/ml for the irradiated samples compared to the unirradiated samples, especially as time progresses.
Figure 5.4a - The Effect of UV Irradiation on Cell Number and Mass Increase in MC14

MC14 was grown as described in the legend to Figure 5.3 except that the C-medium contained 2.5 µg/ml of 12C thymine. Samples of the culture were removed at intervals and particle number and absorbance at 450 nm ($A_{450}$) were measured (see Chapter II).
MC14 was grown and treated as described in the legend to Figure 5.3. Samples were removed at intervals for determination of particle number and $^{14}$C thymine incorporation (see Chapter II).
Unirradiated cpm/ml
Irradiated cpm/ml
Unirradiated cell no.
Irradiated cell no.
When phage \( \lambda \) carrying a mutation in its \( N \) gene infects a sensitive cell it does not grow lytically, rarely integrates into the host chromosome and synthesises little RNA (Skalka et al. 1967; Konrad 1968). It can, however, maintain itself in the cytoplasm like a plasmid (Signer 1969; Lieb 1970) since it can replicate with sufficient frequency to ensure that there is a high probability that each daughter cell will receive a copy of the \( \lambda \) genome at birth. \( \lambda N^- \) mutants thus have some similarities to replicons which have the ability to induce indirectly and it is of interest to determine whether they can do so.

One of the simplest and therefore most attractive explanations for the mechanism of prophage induction is that it is the activity of a repair system which leads to the derepression of the prophage. The difficulty with this hypothesis lies in explaining why the repair system either does not function or does not lead to induction following the introduction of irradiated Hfr DNA or the irradiated DNA of phages T6, \( \Phi 80, \lambda \text{imm}^{434} \) (Rosner et al. 1968), \( \Phi X 174 \) or M13 (Monk 1969) into a \( \lambda \) lysogen. Furthermore, the hypothesis does not provide a simple explanation for the observation that indirect induction is prevented by the pre-existence of a replicon of the same type in a lysogen (Rosner et al. 1968).

It is relatively easy to provide an explanation for the failure of irradiated T6 DNA to induce indirectly. T-even phages are very
complex and upon infection inactivate a number of host functions required for both the transcription and synthesis of host DNA, producing a new series of phage coded functions. Such a radical alteration of the host cell may mean that even if a repair system involved in induction acts on T6 DNA, a later stage in induction or the growth of the induced phage in the cell may be blocked.

It is possible that transferred UV damaged Hfr DNA does not induce because as it enters the lysogenic cell it is rapidly recombined into the host chromosome or broken down and thus never provides an adequate substrate for the repair system involved in induction.

A possible explanation for the failure of irradiated lambdoid phages such as λimm and φ80 to induce is that they might actively inhibit the repair system involved in induction. It is known that the **gam** gene product of λ inhibits the activity of exonuclease V (Unger *et al.* 1972; Unger and Clark 1972) which is required both for efficient genetic recombination and efficient DNA repair. When associated with certain other mutations the **recF** gene product, which is required for prophage induction, controls a recombination mechanism (A.J. Clark, personal communication). There is some preliminary evidence that **recF** mediated recombination may also function at reduced effectiveness in λ infected cells (A.J. Clark, personal communication). Certainly, a **recA** mutation reduces the capacity of a cell to repair UV irradiated λ DNA much less than a mutation inactivating excision repair (Miura and Tomizawa 1968) which could mean that **recA** controlled repair is relatively inefficient at repairing λ DNA. It is possible, therefore, that λ and phage related
to it can actively inhibit the repair mechanism whose action is required for induction. This inhibition is likely to occur to a greatly reduced extent or not at all following infection with a λN- mutant which would not be expected to produce large quantities of inhibitor due to the low level of transcription of most of its genome, in particular the region containing the gam gene (Court and Campbell 1972).

Small single stranded DNA phages such as φX174 and M13 may be very susceptible to the action of repair nucleases when infected into a cell following UV irradiation, so that again it is possible that they might not provide a substrate for the repair system involved in induction (Ono and Shimazu 1967).

The observation which is perhaps the most difficult to explain in terms of a repair hypothesis is that a replicon can be prevented from inducing by the pre-existence in a lysogen of a replicon of the same type. A possible explanation might be, as in the case of irradiated Hfr DNA, that the infecting irradiated DNA is recombined with homologous DNA already in the cell.

The experiments which are described in this Chapter, which involved the use of irradiated λN- DNA, were designed to investigate the following questions. First, given the observation that replicons known to be capable of causing indirect induction have some mechanism for co-ordinating their replication with the host division cycle, can indirect induction be caused by a replicon which has acquired by mutation the ability to be maintained semi-stably in the cell? Second, it is possible that the ability to co-ordinate its replication with the host division cycle may not be important in determining whether a
replicon can cause indirect induction. It has been suggested that many of the replicons which have been tested do not cause indirect induction because they are not susceptible to the action of some repair system. In particular, lambdoid phages may actively inhibit the repair system which is hypothesised to be involved in induction. The $N^-$ mutation would greatly reduce the possibility that a phage function would inhibit the action of a host repair system.

The possibility that the substrate for the hypothesised repair system could be removed by recombination with homologous DNA has also been discussed. Although the infecting irradiated $\lambda$ $N^-$ phage DNA molecules may recombine with the prophage (and with each other) thus possibly eliminating the substrate for the hypothesised repair system, the rate of possible removal of substrate by recombination can presumably be decreased by increasing the multiplicity of infection. For this reason, high multiplicities of infection were generally used in the experiments described below.

**Results**

The Effect of Irradiated $\lambda$ $N^-$ on a $\lambda_{imm}^{434}$ Lysogen

$\lambda$ $N^-$ mutants synthesise $cro$ gene product (Court and Campbell 1972). Eisen et al. (1970) have shown that the $cro$ product can act in trans to regulate the expression of the $cl$ repressor. To prevent its direct action on the prophage repression system a $\lambda_{imm}^{434}$ lysogen was used to determine whether irradiated $\lambda$ $N^-$ can indirectly induce.

The $N^-$ mutant used ($\lambda$ $cl$ sus6 $N$ sus7 sus53) carries an amber mutation in the $cl$ gene in addition to those in the $N$ gene. A $cl$ mutation does not affect the ability of an $N^-$ mutant to replicate
as a plasmid (Lieb 1970) and it removes the possibility that the presence of λ repressor might lower the efficiency with which the inducer can act on the 434 repressor.

The production of λimm\textsuperscript{434} phage following infection by λ N\textsuperscript{−} was measured as the fraction of cells which could produce infective centres when plated on λ lysogenic indicator cells. This method has two advantages over assaying the production of free λimm\textsuperscript{434} phage. First, it minimises any effect which the superinfecting phage might have on reducing the burst size of λimm\textsuperscript{434} (Lieb 1972). Second, a free phage assay would have necessitated using a lysogen resistant to λ to prevent re-adsorption of the λimm\textsuperscript{434} phage released. Since such lysogens would also have been resistant to the λ N\textsuperscript{−} mutant, the experiment would have required the use of phage host range mutants.

The data presented in Figures 6.1 and 6.2 show that infection with irradiated λ N\textsuperscript{−} can stimulate growth of λimm\textsuperscript{434} in up to 25% of the infected lysogens. Maximum stimulation occurs when W3350 (λimm\textsuperscript{434}) is infected with about 15 λ N\textsuperscript{−} phages per cell (Figure 6.1) and when the λ N\textsuperscript{−} has been irradiated with 750 ergs mm\textsuperscript{−2} (Figure 6.2).

Similar results were obtained whether stimulation of phage production was measured by the production of phage capable of plating on a C600 (λ) indicator strain or on a W3350 (λ) indicator strain, as is shown in Figure 6.3. Since C600 contains an amber suppressor, a phage will form a plaque on it even if it carries an amber mutation in its N gene. This is not the case after infection of W3350, which does not contain an amber suppressor.

Infection with unirradiated λ N\textsuperscript{−} also leads to some stimulation of λimm\textsuperscript{434} growth. This is particularly noticeable in Figures 6.1 and
6.2. This may be due partly to recombination between $\lambda N^-$ DNA and the prophage, and partly to a weak interaction between the $\lambda cro$ gene product and the 434 immunity system. There is evidence that the 434 antirepressor can weakly antagonise the $\lambda$ repression system (Calef et al. 1971) and the converse may also be true.

The Effect of Irradiated $\lambda N^-$ on a Mutant of $\lambda^434$ not Directly Inducible by UV Irradiation

The observation that irradiated $\lambda N^-$ can stimulate the growth of $\lambda^434$ does not prove that this stimulation is a consequence of indirect induction. To prove this it is necessary to show that irradiated $\lambda N^-$ cannot stimulate the growth of a $\lambda^434$ mutant which is not directly inducible by UV irradiation.

A $\lambda^434$ ind$^-$ mutant was isolated by the method described in Chapter II and W3350 was lysogenised with it. The plaques formed on W3350 by this phage mutant were similar in size and turbidity to those formed by $\lambda^434$. The phage mutant which was used was chosen because it was spontaneously induced with about the same efficiency as $\lambda^434$. This suggests that the phage is able to excise from the host chromosome and to grow in the cell and that it is non-inducible because it is not derepressed following UV irradiation.

UV induction curves of W3350 ($\lambda^434$) and W3350 ($\lambda^434$ ind$^-$) are shown in Figure 6.4.

Although W3350 ($\lambda^434$ ind$^-$) cannot be induced by direct UV irradiation, $\lambda^434$ ind$^-$ can be stimulated to grow lytically almost as well as $\lambda^434$ by irradiated $\lambda N^-$ (Figure 6.5). It seems therefore that at least the majority of the $\lambda^434$ growth stimulated by $\lambda N^-$ is
not due to indirect induction and that irradiated \( \lambda N^- \), if it does induce, does so with low efficiency.

It is interesting that the \( \lambda \text{imm}^{434} \text{ind}^- \) lysogens can be stimulated to form infective centres somewhat better than \( \lambda \text{imm}^{434} \) lysogens by unirradiated \( \lambda N^- \). If, as suggested earlier, this stimulation of the growth of \( \lambda \text{imm}^{434} \) by unirradiated \( \lambda N^- \) is partly due to a weak interaction between the \( \lambda \text{cro} \) product and the 434 repression system, it could be that a mutation in the 434 repressor which prevents UV induction also makes it more sensitive to the antirepressor action of the \( \lambda \text{cro} \) gene product.

The Effect of Irradiated \( \lambda N^- \) on a \( \lambda \text{imm}^{434} \) lysogen mutant at \( \text{recA} \)

If the stimulation of \( \lambda \text{imm}^{434} \) phage production by irradiated \( \lambda N^- \) is not due to indirect induction, the most probable explanation is that it is due to recombination between the prophage and the irradiated superinfecting phage DNA. The recombination frequency of \( \lambda \) DNA is known to be markedly enhanced if it contains UV photoproducts (Jacob and Wollman 1955).

In order to test this hypothesis, the stimulation of growth of \( \lambda \text{imm}^{434} \) was compared using a pair of lysogenic strains isogenic with the exception of the region of the chromosome carrying the \( \text{recA} \) gene.

A \( \text{sup}^+ \) \( \text{recA} \) lysogen was constructed by mating \( \text{W3110 thyA Sm}^R \) with \( \text{KL16-99 recA} \) for 10 minutes and selecting \( \text{thy}^+ \text{Sm}^R \) recombinants. These recombinants were tested for their UV and X-ray sensitivity. A UV and X-ray sensitive and a UV and X-ray resistant strain were chosen for use and lysogenised with \( \lambda \text{imm}^{434} \). These strains were streptomycin resistant and the penicillin technique was employed in the infective centres assay instead of the streptomycin overlay technique usually
employed in the work described in this thesis (see Chapter II).

As can be seen from Figure 6.6, the stimulation of \( \lambda_{imm}^{434} \) phage production by irradiated \( \lambda^N \) is greatly reduced in a \( \text{recA} \) lysogen, suggesting that the phage are formed by recombination between \( \lambda^N \) and the prophage.

The \text{red} genes of the phage are unlikely to play a major part in recombining \( \lambda^N \) DNA with the prophage since, in the absence of \( N \) gene product they will not be expressed efficiently (Court and Campbell 1972). The host \text{rec} system is normally rather inefficient at recombining \( \lambda \) DNA because \( \lambda \) produces the \text{gam} gene product which inhibits exonuclease \( V \), the product of the \text{recB} and \text{recC} genes (Unger et al. 1972; Unger and Clark 1972). However, since \( \lambda^N \) mutants fail to produce the \text{gam} gene product (Court and Campbell 1972) the host \text{rec} system should function efficiently in recombining \( \lambda^N \) and prophage DNA.

An interesting feature of the experiment shown in Figure 6.6 is the comparatively high level of spontaneous induction observed in the \( \lambda_{imm}^{434} \) lysogen mutant at \text{recA}. This occurs at about 10\% of the level observed in the \text{rec}+ strain. This is much higher than the level reported for \( \lambda \) lysogens of \text{recA} mutants (Brooks and Clark 1967).

The observation that unirradiated \( \lambda^N \) can stimulate \( \lambda_{imm}^{434} \) growth in a \text{recA} lysogen supports the idea, discussed above, that this stimulation is in part due to causes other than recombination, possibly to a weak interaction between the 434 repression system and the \( \lambda \text{cro} \) antirepressor.
Discussion

The results reported in this Chapter demonstrate that irradiated λ N DNA cannot indirectly induce with measurable efficiency. They suggest that the fortuitous acquisition by mutation of the ability to be maintained semi-stably in the cell does not confer on a replicon the ability, when irradiated, to induce indirectly. These experiments thus support the concept that replications which can indirectly induce require some specific mechanism for co-ordinating their replication with the host division cycle.

In the introduction to this Chapter, the hypothesis was considered that induction results from the activity of a repair system and that for various reasons this repair system does not act on all types of damaged DNA. In the case of irradiated lambdoid phage DNA, it was suggested that expression of the infecting phage genomes leads to an active inhibition of the repair system.

In the light of the experiments reported here, this possibility must be considered very unlikely unless the inhibitor can be effectively produced in the absence of N gene product.
Figure 6.1 - The Effect of Multiplicity of Infection (MOI) on the

Stimulation of Growth of λimm<sup>434</sup> by irradiated λ

<i>cIsus6 Nsus7sus53</i>

W3350 (λimm<sup>434</sup>) was shaken vigorously in TBMM at 37°C until the absorbance at 450 nm was 0.25. A sample of the culture was washed and resuspended in LB, concentrating the cells about fourfold in the process. λ <i>cIsus6 Nsus7sus53</i> was diluted appropriately in LB and, where necessary, UV irradiated with 1000 ergs mm<sup>-2</sup>. 0.2 ml portions of the cells were then incubated at 37°C with 0.2 ml aliquots of phage (or 0.2 ml of LB to measure spontaneous induction). After 20 minutes the mixture was diluted in LB and 0.1 ml samples were plated with C600 Sm<sup>R</sup> (λ) indicator cells on LTA plates. The plates were subsequently overlaid with streptomycin as described in Chapter II. λ <i>cIsus6 Nsus7sus53</i> was titred on C600 indicator cells. The open circle shows the fraction of W3350 (λimm<sup>434</sup>) lysogens forming infective centres following infection with unirradiated λ<sup>N</sup> at a high multiplicity of infection.
Figure 6.2 - The Effect of the UV Dose Given to λ cIsus6 Nsus7sus53 on its ability to stimulate the growth of λimm

The experiment was similar to that described in the legend to Figure 6.1 except that the cells were infected with a constant multiplicity of phages (10), which had received varying UV doses.
Spontaneous induction
Figure 6.3 - The Ability of the Phage Released after Infection of W3350 (λimm\textsuperscript{434}) with irradiated λ N\textsuperscript{-} to Form Plaques on C600 Sm\textsuperscript{R} (λ) and W3350 Sm\textsuperscript{R} (λ).

The experiment was similar to that described in the legend to Figure 6.1 except that where appropriate the phage was irradiated with 750 ergs mm\textsuperscript{-2}. After λ N\textsuperscript{-} had been adsorbed to it, W3350 (λimm\textsuperscript{434}) was plated with both C600 Sm\textsuperscript{R} (λ) and W3350 Sm\textsuperscript{R} (λ) indicator strains.


% Lysogens forming infective centres

MOI

- Irradiated $\lambda N^-$
- Unirradiated $\lambda N^-$

$W3350(\lambda)$
$C600(\lambda)$
Figure 6.4 - UV Induction Curves of W3350 (λimm434) and W3350 (λimm434 ind−)

W3350 (λimm434) and W3350 (λimm434 ind−) were grown with shaking aeration in TBMM at 37°C until the absorbance at 450 nm was 0.3. A sample of each culture was washed and resuspended in LB. The cells were irradiated with an appropriate dose of UV and plated with C600 SmR (λ) indicator cells. Streptomycin was added to the plates after 2½ hours at 37°C as described in Chapter II.
% Induction

UV dose ergs mm$^{-2}$

△ W3350 (λimm 434)
△ W3350 (λimm 434 ind−)
W3350 (λimm$^{434}$) and W3350 (λimm$^{434}$ ind$^{-}$) were grown and treated as described in the legend to Figure 6.1. They were infected with λ$^{N^-}$, which had been irradiated, where appropriate, with 750 ergs mm$^{-2}$.}

Figure 6.5 - The Effect of Irradiated λ$^{N^-}$ on W3350 (λimm$^{434}$) and W3350 (λimm$^{434}$ ind$^{-}$)
Figure 6.6 - The Effect of a recA Mutation on the Ability of a
\( \lambda N^- \) Mutant to Stimulate \( \lambda imm^{434} \) Phage Production

\( \text{W3110 recA} (\lambda imm^{434}) \) and \( \text{W3110 rec}^+ (\lambda imm^{434}) \) were grown and treated as described in the legend to Figure 6.1 except that the phages were irradiated with 750 ergs mm\(^{-2}\) and, after adsorption of the \( \lambda N^- \), the cells were plated in LSA containing 100 \( \mu g/ml \) of penicillin on LTA plates also containing 100 \( \mu g/ml \) of penicillin. The indicator strain used was C600 Pen\(^R\) (\( \lambda \)).
CHAPTER VII

THE MECHANISM OF PROPHAGE INDUCTION RECONSIDERED

In Chapter I, a number of general hypotheses were considered as possible explanations for the mechanism of prophage induction. Since the work described in this thesis does not suggest a clear alternative model, these hypotheses can now be considered in more detail.

(1) **Induction Is Caused by the Accumulation of Some Component Involved in the Initiation of Rounds of DNA Replication**

The experiments described in Chapter IV show that the age of optimal induction of lysogens growing with a series of different growth rates does not correlate with the age at which a round of DNA replication is initiated and are clearly inconsistent with the model. Furthermore, the hypothesis provides no obvious explanation for the behaviour of the mutant T44 or for the non-inducibility of *recA*, *lex*, *zab* and *recF* mutants and is inconsistent with the data of Monk and Gross (1971) unless additional assumptions are made, as discussed in Chapter I.

(2) **Induction Is Caused by the Action of Some DNA Repair Mechanism**

This hypothesis could be consistent with the observation that cells of different ages vary in their sensitivity to UV induction. It is unlikely, though possible for reasons discussed in Chapter 5 that induction is caused by the preferential action of a repair enzyme at a certain stage in the DNA replication cycle. Such a hypothesis does not provide a simple explanation for the change in the inducibility of
a population of lysogens following a shift-up. However, the difficulties of interpreting this experiment have already been discussed in Chapter V.

The basic objection to the hypothesis that induction results from the activity of a repair system is that it does not provide a simple explanation for the observation that only certain replicons cause indirect induction. An attempt was made, as described in Chapter VI, to invalidate the hypothesis that indirect induction requires the introduction into a lysogen of an irradiated replicon which possesses a specific mechanism for co-ordinating its replication with the host division cycle. However, irradiated λ NI, despite the fact that it could be recombined by a recombination system requiring the recA gene product could not induce a λimmreff prophage efficiently. This does not support the hypothesis that induction solely requires the action of a repair enzyme.

A number of variations on the basic hypothesis that induction is caused by the action of a repair mechanism have been suggested in the literature. For example, Benbow et al. (1973) have suggested that induction could be caused by recombination between UV irradiated DNA and the prophage. This hypothesis is based upon the proposition that following transfer into a recipient, irradiated F DNA contains pyrimidine dimers opposed by gaps in the newly synthesised complementary strand (George and Devoret 1971) and such structures in chromosomal DNA are known to induce genetic exchanges efficiently (Rupp et al. 1971). A study of indirect curing by an irradiated F 1ac, using a defective prophage whose derepression tends to lead
to curing, revealed that curing preferentially occurred in those cells which form \( \text{lac}^+ \text{F}^- \) recombinants. In other words, curing tended to occur in those cells in which recombination had taken place between the damaged episome and the chromosome.

However, there are a number of observations which are not easily reconciled with the hypothesis proposed by Benbow et al. (1973). First, it does not explain indirect induction satisfactorily. There should be very extensive recombination between, for example, transferred irradiated Hfr DNA and the chromosome of the recipient cell, yet induction occurs inefficiently, if at all, in this system. Also, evidence presented in this thesis (see Chapter VI) suggests that \( \text{recA} \) mediated recombination occurs between an inducible prophage and irradiated \( \lambda \text{N}^- \) DNA without induction taking place. Second, the hypothesis is not in good agreement with the properties of mutants which have been shown to have altered inducibility. Recombination is not stimulated in T44 under inducing conditions, as measured, for example, by an interaction between an \( \text{F lac}^+ \) episome and the chromosome leading to chromosome mobilisation (Castellazzi et al. 1972a).

Furthermore, \( \text{lex}, \text{zab} \) and \( \text{recF} \) mutants are not markedly recombination deficient unless, in the latter case, associated with other mutations, although they are deficient in their inducibility. However, it is possible that the products of the \( \text{lex}, \text{zab} \) and \( \text{recF} \) genes could participate in the recombination of UV damaged DNA. Third, it is difficult to reconcile the experiments of Monk and Gross (1971) (see also Chapter V), which show that induction can occur following irradiation of a \( \text{dnaA} \) lysogen after 75 minutes incubation at 42°C with any hypothesis.
linking induction and recombination. It has been shown that when dnaA-46, growing at 30°C with a generation time of 90 minutes, is placed at the non-permissive temperature, there is doubling in viable cell number, despite the fact that there is only a 30% increase in DNA content (E. Tresguerres, personal communication). This implies that dnaA mutants at the time of irradiation in the experiments described by Monk and Gross (1971) and in Chapter V will contain a single unreplicated chromosome, with no opportunity for recombination.

A second variation on the hypothesis that induction is caused by the action of a repair mechanism is that of Castellazzi et al. (1972b). These workers argue that induction may depend upon the activity levels of the gene products involved in DNA repair. They suggest that these levels are increased after tif-l expression in T44 and after UV irradiation and other inducing treatments, possibly as a result of DNA lesions. They further suggest that these gene products can cause λ induction in the absence of damaged DNA when their activity levels are increased following tif-l expression. To summarise, this hypothesis implies that inducing treatments first activate or induce a repair system and this leads to derepression of the prophage.

The phenomenon of the increased survival and mutagenesis of irradiated phage grown on T44 following the expression of the temperature sensitive mutation in this strain (see Chapter I) bears a striking resemblance to the phenomenon of ultraviolet reactivation (UVR) (Weigle 1953). UVR is the term given to the enhanced survival and mutagenesis of an irradiated phage resulting from infection of a
lightly irradiated host. Both UVR and the enhanced repair due to tif-l expression require active recA*, lex* and zab* gene products (Miura and Tomizawa 1968; Defais et al. 1971; Castellazzi et al. 1972b).

To propose that prophage induction occurs as a result of the activation of the repair system involved in UVR is to suggest that this repair system is activated by irradiated replicons which can cause indirect induction. This is not in good agreement with the observation that one can enhance the survival of irradiated P1 phage by UV irradiation of the cell prior to infection (Kerr and Hart 1973). Since P1 can induce λ (Rosner et al. 1968) it should also be able to activate the repair mechanism postulated in the hypothesis under discussion and its own survival should not be enhanced by prior irradiation of the cell. This argument is weakened, however, by the observation that irradiated P1 induces λ with a rather low efficiency (Rosner et al. 1968) so that its hypothesised activation of the repair system may be incomplete.

In summary, it is difficult to construct a simple direct hypothesis linking DNA repair and prophage induction. The difficulty is further underlined by the behaviour of Itd-1 mutants, which are non-inducible by either thymine deprivation or UV irradiation but which are as resistant as wild-type to the killing action of UV and X-irradiation (Devoret et al. 1972). Furthermore, Itd-1 mutants are not defective in UVR (Devoret et al. 1972).

It may be that repair deficient mutants have an altered inducibility because of the secondary effects which such mutations
may have on cell physiology. Some of them have secondary effects on cell division (see Chapter I) which, as discussed below, may be involved in induction. The observation that recA, lex and zab can prevent the filamentation of T44 under inducing conditions in the absence of compelling evidence for a disturbance to DNA metabolism, is consistent with the hypothesis that such mutants affect induction because of some secondary effect on cell physiology.

(3) Induction is related to the Inhibition of Cell Division

As has been discussed in Chapter IV, the observation that lysogens are most readily inducible between 21 and 30 minutes before division at a variety of growth rates and least readily inducible at the time of cell division could be consistent with the hypothesis that induction is related to the inhibition of cell division. The observation of the effect which UV irradiation has on cell division in a dnaA mutant is also consistent with this hypothesis. The data showing the increase in inducibility of a population of lysogens after a nutritional shift-up are in less obvious agreement with the model.

Some more detailed aspects of the hypothesis that prophage induction and the inhibition of cell division are linked can now be considered.

(a) Induction is a Direct Consequence of the Inhibition of Cell Division

The evidence against this hypothesis has been discussed in Chapter I and will not be reconsidered here. The data on the effect of a shift-up on the inducibility of a population of lysogens, reported in Chapter V, where the frequency of septum formation does not change
for at least 60 minutes after the shift, whereas the inducibility of the population rises immediately, can be reconciled with this hypothesis if it is assumed that division takes longer to recover from the inhibitory effect of irradiation under these conditions.

(b) **Induction results from the Attainment of a Critical Cell Size Relative to the DNA Content of the Cell**

It may be that induction depends not so much upon the inhibition of septum formation as upon the cell reaching some critical size relative to its DNA content or, more specifically, to the number of λ genomes it carries. This might be achieved either as a result of the inhibition of cell division following a disturbance to DNA metabolism or following a shift to a nutritionally richer medium where the decrease in the DNA to mass ratio (Kjeldgaard et al. 1958; Cooper 1969) would become very marked if DNA synthesis was inhibited. This could explain the rapid change in the inducibility of the population which is observed.

The principal objections to this hypothesis are first, the behaviour of the mutant T44, in which there is no evidence for an altered DNA to mass ratio under inducing conditions (Kirby et al. 1967). Second, although as discussed in Chapter I, there is evidence that an irradiated ColI factor can inhibit cell division in a recipient cell (Monk 1969) there is no evidence that the damaged plasmid has a marked effect on the rate of chromosome DNA synthesis. Third, the hypothesis does not accommodate the observation that dnaA (λ) lysogens do not apparently become spontaneously induced after prolonged incubation at the non-permissive temperature, despite the increase in size of many of the cells (Hirota et al. 1968). Fourth, as discussed
in Chapter I, there is some doubt whether protein synthesis is required for induction.

(c) Following UV Irradiation, the Inhibition of Cell Division and Prophage Induction Both Result from a Closely Related Series of Events but They Are Not Causally Related

This hypothesis is in good agreement with the available evidence. The most difficult results to reconcile with it are the data from the shift-up experiment reported in Chapter V. However, cell division is a complex phenomenon whose regulation is far from understood. There is evidence that some proteins involved in cell division accumulate independently of the DNA replication cycle once it has been initiated (Jones and Donachie 1973) and following a nutritional shift-up these or related components may accumulate rapidly and result in efficient induction once the cells have been irradiated. It is of interest that cell division can be inhibited in lon mutants by shifting them to a nutritionally richer medium (Walker and Smith 1970). This may suggest that cells have temporary difficulties in regulating division during a shift-up and renders it difficult to interpret the results of transition experiments.

The hypothesis under discussion depends upon the assumption that cell division can be inhibited by the introduction into a lysogen of an irradiated replicon which possesses a mechanism for co-ordinating its replication with the host division cycle, provided that the lysogen does not already harbour a replicon of the same type. This assumption is supported by the demonstration that an irradiated ColI factor can inhibit division in a recipient cell (Monk 1969) but this
observation clearly needs to be extended by observations on the effects of other irradiated replicons on division in recipient cells.

As will have become apparent from the discussion to Chapter V, it is difficult to construct a simple hypothesis to explain the mechanism of inhibition of cell division following treatment with UV. It is not certain whether UV inhibits division by interfering with the normal regulatory mechanism controlling cell division or whether its inhibitory effect on division is independent of this mechanism. It seems unlikely that this problem can be resolved without a better understanding of the control of cell division.

It is interesting, however, to speculate on the nature of the possible connection between the inhibition of cell division and prophage induction. Recent evidence suggests that the λ cro antirepressor, which may act as a repressor at similar and possibly identical operators to the cI repressor (Oppenheim, personal communication) though with quantitatively different results on the level of transcription, functions inefficiently in a colicin-tolerant mutant of E.coli which has an altered membrane protein (Oppenheim, Honigman and Oppenheim, to be published). Further, Walker et al. (1973) have suggested that cI repressor synthesis or function may be interfered with in lon mutants which have been shown to have an altered membrane protein (Leighton 1972).

It is possible that following treatment with an inducing agent membrane changes occur which lead both to an inhibition of cell division and an interference with the cI repression system. Inouye and Pardee (1970) have shown that increased production of a membrane protein X occurs when cell division is inhibited following treatment with agents
such as UV and nalidixic acid and following thymine starvation. The increased production does not occur if DNA synthesis is inhibited in a recA mutant (Inouye 1971). Furthermore, it does not accumulate if a dnaB mutant (te27) is incubated at 41°C, under which conditions DNA synthesis ceases immediately but the cells continue dividing. In contrast, increased production of membrane protein X can be observed if division is inhibited under these conditions by the addition of nalidixic acid (Inouye and Pardee 1970; Inouye 1972). However, changes in relative amounts of membrane proteins have also been detected when dnaA mutants are shifted from the permissive to the non-permissive temperature (Shapiro et al. 1970; Siccardi et al. 1971; Lazdunski and Shapiro 1973), so that these results must be interpreted with caution.

The dnaB mutant which Noack and Klaus (1972) reported could be induced by shifting to the restrictive temperature when lysogenic was originally described by Bonhoeffer (1966) and was shown by Clark (1968a) to stop dividing upon incubation at the restrictive temperature. It would be of interest to determine whether the dnaB mutant described by Inouye (1969) which can continue dividing at 41°C can be induced by incubation at this temperature. This is currently under investigation.

Conclusions

A number of hypotheses proposed to explain the mechanism of prophage induction have been evaluated in the light of published evidence and the experiments described in this thesis. It has been suggested that the hypothesis most consistent with the available evidence is that induction results from the accumulation or disappearance of a cellular component which is involved in inhibiting cell
division following damage to a replicon which can normally co-ordinate its replication with the division cycle. It has been further suggested that the common intermediate which links the inhibition of cell division and the derepression of the prophage may be an alteration in the host cell membrane.

It would be misleading, however, to conclude that induction can only be explained by linking it with the inhibition of cell division. The observations which have been made on which replicons can cause induction when damaged and the variation in inducibility through the cell cycle of synchronous populations of lysogens are, however, most simply explained on the hypothesis that the primary event leading to induction is some form of disturbance to the cell cycle.

Finally, it should be pointed out that many repressors and indeed other proteins in *E. coli* may be affected by the inducer which leads to the derepression of λ. Great caution must therefore be applied in linking alterations observed in the behaviour of cells under inducing conditions with prophage induction, whether these alterations are increased repair activity or the inhibition of cell division.


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Treatment of bacteriophage lambda (λ) lysogens of Escherichia coli with various physical and chemical agents leads to induction. This thesis reports some investigations into the mechanism of induction, in particular following UV irradiation.

Lysogens of different ages were found to vary in the efficiency with which they are induced by small doses of UV irradiation, being least inducible at division and most inducible at a time which coincides approximately with the time when rounds of DNA replication are thought to be terminated.

One can indirectly induce a λ prophage by introducing into a lysogen an entire irradiated plasmid. Experiments are described showing that a λN− mutant is unable to indirectly induce a prophage when irradiated. This suggests that the fortuitous acquisition by mutation of the ability to be maintained semi-stably in the cell in a non-integrated state is not sufficient to give a replicon the ability, when irradiated, to indirectly induce.

Evidence is also presented in support of the correlation which exists between treatments leading to induction and those leading to the inhibition of cell division.

These results have been considered in terms of three general hypotheses.

1. Induction is caused by the accumulation of some component involved in the initiation of rounds of DNA replication. This is clearly inconsistent with data showing the age at which lysogens are most readily inducible.

2. Induction is related to the activity of some DNA repair system. This hypothesis is consistent with much of the data presented in this thesis but is difficult to reconcile in its simplest form with the observation that only certain types of irradiated DNA indirectly induce.

3. Induction is related to the inhibition of cell division. This hypothesis is consistent with published work and receives support from data presented in this thesis.